Skeletal Growth and Life History Evolution in Wild and Domesticated Mammals

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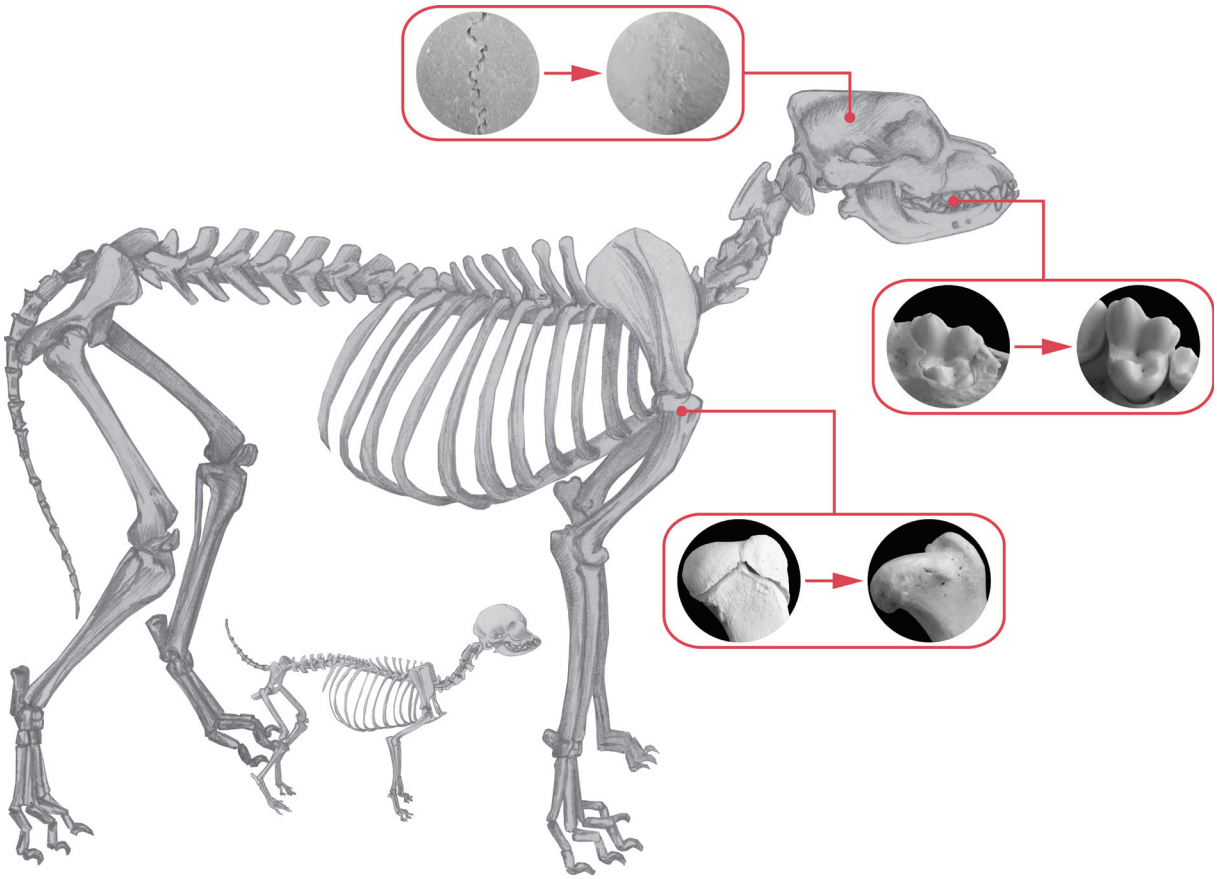
To my family

Skeletal Growth and Life History Evolution in Wild and Domesticated Mammals



Madeleine Geiger
Universität Zürich, 2015

Title page image:

Extreme size variation within one species, exemplified by a St. Bernard dog and a Chihuahua.

The markers of dental and skeletal growth which I was investigating throughout my PhD thesis are illustrated: closure of the cranial sutures and synchondroses, eruption of the permanent dentition into occlusion, and closure of the postcranial growth plates.

Artwork by Madeleine Geiger, 2015, inspired by the Natural History Museum Bern.

Contents

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Summary

Not much is known about the generation of morphological disparity by means of differential patterns of growth during postnatal ontogeny in mammals. This PhD thesis aims at elucidating aspects of growth, generation of variability and disparity, and their association to life history traits by investigating specific markers of growth (tooth eruption, growth plate closure, and suture closure) in mammals in general and domestic dogs in particular. Domestic dogs are tremendously variable, although one single species, and relatively well investigated. They thus comprise an ideal study subject for addressing these questions. The study of skeletal and dental growth and alterations of skeletal morphology throughout the domestication of dogs, in conjunction with knowledge about variation in life history traits, can not only improve our understanding of the ontogeny and domestication of dogs, but also shed light on the mechanisms of domestication in general and the evolution of other species that live and evolve under a special selective pressure leading to rapid evolution, e.g. on islands.

The first chapter deals with heterochronic patterns (shifts in timing) of growth plate closure in mammals which display a broad spectrum of limb specialisations, coupled with different locomotor strategies and habitat occupation. A common general pattern of growth plate closure sequence was found, but one that is universal neither among species nor in higher-order taxa. Observed patterns indicate that growth plate closure sequence is more strongly influenced through phylogenetic inertia than through locomotor adaptations. It was also found that heterochronic shifts in the growth plate closure sequence of marsupials occur with a higher rate than in placentals, which is in contrast with the more limited variation in timing and morphospace occupation typical for marsupial development. Moreover, unlike placentals, marsupials maintain many epiphyses separated throughout life, which probably represent the derived state.

The second chapter concerns patterns of growth plate closure and tooth eruption in domestic dogs. It has been argued that the domestication process has altered the sequence of dental maturity, skeletal maturity, and sexual maturity due to altered environmental conditions. The aim of this study was to test this hypothesis by investigating a comprehensive sample of domestic dog skeletons and teeth. The analyses show that there is no change in the sequence of dental, skeletal, and sexual maturity in dogs due to domestication and that, despite their great variability, domestic dogs are wolf-like in terms of the here investigated processes of somatic and sexual maturation.

In the third chapter, it was investigated whether bulldog-like domestic dog breeds, which are characterised by a relatively short and broad skull with a dorsally rotated rostrum, exhibit similar patterns of suture and synchondrosis closure than exhibited in some human syndromes associated with a ‘bulldog-like’ small face and additionally premature fusion of certain craniofacial sutures and cranial base synchondroses, e.g., achondroplasia, Apert syndrome, and Crouzon syndrome. Comparisons of the relative amount of closing and closed sutures and synchondroses in adult individuals showed that bulldog-type domestic dog breeds have significantly higher closure scores than non-bulldog-type breeds and that domestic dogs have significantly higher closure scores than the wolf. These results indicate that similar mechanisms might act on the generation of the bulldog-type skull conformations in humans and domestic dogs, but at the same time emphasise the importance of biomechanical influences on patterns of suture closure in domestic dogs.

The fourth chapter examines the controversial hypothesis of an association of retardation of growth (heterochrony: paedomorphosis) with the generation of the typical skull shape in domestic dogs, with the latter being ‘paedomorphic wolves’. We conducted

the first geometric morphometric analysis of ontogenetic series of dogs from different historical periods of domestication (modern, premodern, prehistoric), serving to provide a comprehensive test of the paedomorphosis hypothesis. The mandible was also investigated, to examine the complete picture of morphological disparity of skull shape. We found that adult cranial and mandibular shape in some, mainly premodern domestic dogs, is similar to wolves, but does not intersect with their ancestral ontogenetic trajectory. In cases where ontogenetic trajectories of wolves and domestic dogs overlap, these trajectories are similar to each other, indicating similar patterns of growth. Therefore, we did not find evidence for heterochrony in the investigated groups and conclude that domestic dogs are not paedomorphic wolves.

Keywords

Ontogeny, heterochrony, mammal, domestication, dog, growth plate, suture, synchondrosis, tooth, cranium, mandible, skeleton

Zusammenfassung

Über die Entstehung morphologischer Variationen in Säugetieren durch unterschiedliche nachgeburtliche Wachstumsmuster ist nicht viel bekannt. Das Ziel dieser Doktorarbeit ist Aspekte des Wachstums, der Entstehung von Variation und deren Verbindung zum Lebenszyklus zu erläutern. Dazu wurden spezielle Wachstumsmarker (Zahneruption, Epiphysenfugenverschluss und Suturenverschluss) in Säugetieren und speziell in domestizierten Hunden untersucht. Domestizierte Hunde (Haushunde) sind hoch variabel und relativ gut erforscht weshalb sie ein ideales Studienobjekt zur Untersuchung dieser Fragen darstellen. Die Erforschung des Skelett- und Zahnwachstums in Zusammenhang mit Variationen des Lebenszyklus kann aber nicht nur unser Verständnis über die Entwicklung und Domestikation von Hunden verbessern. Sie dient auch der Aufklärung von Mechanismen der allgemeinen Domestikation und der Evolution von Arten unter domestikationsähnlichen, speziellen Selektionsdrücken. Ein Beispiel dafür ist die Evolution von Inselpopulationen.

Das erste Kapitel dieser These bespricht Heterochronien (Verschiebungen im zeitlichen Ablauf der Entwicklung) im Verschluss von Epiphysenfugen in Säugetierarten mit einem breiten Anpassungsspektrum an verschiedene Fortbewegungsformen. Die Ergebnisse zeigten ein gemeinsames, generelles Muster in der Reihenfolge des Epiphysenfugenverschlusses. Dieses ist jedoch zwischen den verschiedenen Säugetierarten und zwischen höheren Taxa nicht komplett identisch. Des Weiteren scheint die Reihenfolge des Epiphysenfugenverschlusses eher durch stammesgeschichtliche Verwandtschaft als durch Anpassungen an eine bestimmte Art der Fortbewegung beeinflusst zu sein. Als Besonderheit ist anzumerken, dass Heterochronien in Beuteltieren mit einer höheren Rate auftreten als in Plazentatieren. Dieses Resultat steht im Gegensatz zu der für Beuteltiere typischen, limitierten Variationsbreite von zeitlichen Abläufen in der Ontogenie sowie möglichen Morphologien. Im Gegensatz zu Plazentatieren bleiben in Beuteltieren zudem viele Epiphysenfugen während des gesamten Lebens offen. Dies ist womöglich eine abgeleitete Eigenschaft der Beuteltiere.

Das Zweite Kapitel behandelt Muster des Epiphysenfugenverschlusses und der Zahneruption in Haushunden. In der Literatur wurde argumentiert, dass durch Veränderungen in der Umwelt während des beginnenden Domestikationsprozesses die Reihenfolge von dentaler, ossealer und sexueller Reife verschoben wurde. Das Ziel dieser Studie war die Untersuchung dieser Hypothese mit einer umfassenden Stichprobe von Haushund- und Wolfskeletten. Die Analysen zeigen, dass durch die Domestikation keine Verschiebungen im Erlangen von dentaler, ossealer und sexueller Reife stattgefunden haben und dass Hunde in diesen Eigenschaften wolfsähnlich sind.

Im dritten Kapitel wurden die Muster des cranialen Suturen- und Synchondrosenverschlusses in bulldoggen-ähnlichen Haushunderassen untersucht. Solche Rassen zeichnen sich durch einen relativ kurzen, breiten Schädel und eine dorsal geknickte Schnauze aus (z.B. Französische Bulldogge). In Menschen sind einige Syndrome bekannt, bei denen ein bulldoggen-ähnliches, kleines Gesicht mit einem verfrühten Verschluss von Sutruen und Synchondrosen gekoppelt ist (z.B. Achondroplasie, Apert- und Crouzon-Syndrom). Das Ziel dieser Studie war die Überprüfung der Frage, ob bulldoggen-ähnliche Hunderassen ähnliche Suturenverschlussmuster zeigen wie Menschen mit diesen Syndromen. Vergleiche der relativen Zahl sich verschliessender und verschlossener Suturen und Synchondrosen in ausgewachsenen Individuen haben gezeigt, dass bulldoggen-ähnliche Haushunde eine signifikant höhere Schliessungsr-Rate aufweisen als andere Rassen. Ausserdem weisen Haushunde im Allgemeinen eine höhere Schliessungs-Rate auf als Wölfe. Weiterhin

zeigen diese Resultate, dass für die Bildung von bulldoggen-ähnlichen Schädelmerkmalen in Menschen und Haushunden wahrscheinlich ähnliche Mechanismen verantwortlich sind. Zudem wird gleichzeitig auch die Wichtigkeit von biomechanischen Einflüssen auf die Muster des Suturenverschlusses in Haushunden aufgezeigt.

Die vierte Studie untersucht eine kontroverse Hypothese über die Verbindung zwischen einer Retardation des Schädelwachstums (Heterochronie: Pädomorphose) und dem Auftreten der Haushundetypischen Schädelform. Hunde werden in diesem Zusammenhang oft als pädomorphe, verjugendlichte Wölfe bezeichnet. Für diese Dissertation wurde die erste geometrisch morphometrische Studie durchgeführt, die Haushunde aus verschiedenen historischen Epochen der Domestikation (modern, vormodern, prähistorisch) miteinander vergleicht und zu einem umfassenden Test dieser Pädomorphie-Hypothese geeignet ist. Der Unterkiefer wurde in diesem Zusammenhang ebenfalls untersucht, um das Bild der morphologischen Unterschiedlichkeit der Schädel zu vervollständigen. Die Resultate zeigen, dass die Form der Schädel und Unterkiefer in manchen, vornehmlich vormodernen, adulten Haushunden ähnlich derjenigen der Wölfe ist. Die Schädelformen dieser Haushunde sind allerdings nicht kongruent mit der ontogenetischen Trajektorie des Wolfes. In den Fällen, in denen die ontogenetischen Trajektorien von Haushunden und Wölfen überlappen, sind diese kongruent. Dies deutet darauf hin, dass auch die Wachstumsmuster gleich sind und deshalb keine Heterochronie nachgewiesen werden kann. Es kann deshalb gefolgert werden, dass Haushunde keine pädomorphen Wölfe sind.

Schlüsselwörter

Ontogenie, Heterochronie, Mammalia, Domestikation, Haushund, Epiphysenfugen, Sutur, Synchondrose, Zahn, Cranium, Mandibel, Skelett

INTRODUCTION

Introduction

Whilst walking in urban areas these days one cannot avoid recognising the ubiquity of domestic dogs. They are so close to us humans that they are hardly appreciated by many during everyday life, although the multitude of the varieties is astonishing and their appearance is occasionally most unusual. It seems natural to us that a tiny Chihuahua, a stocky pug, a gracile Saluki, and an enormous St. Bernard all belong to the same kind of organism, or species. And yet, these organisms represent an extraordinary case of phenotypic disparity. If becoming aware of this fact, one might wondering which processes may have led to the observed variability within just one species and what the course of the observed divergence might has been. During my PhD, these were the greater questions which I was interested in and I hope that my work will help elucidating some aspects of them.

In the following paragraphs of this chapter I am introducing the nature of domestication per se and the domestication of dogs in particular. Most importantly, I would like to emphasise that domestication is not a process of artificial degeneration of nature, but a peculiar case of adaptation to a niche which is created as a function of the interaction between two species (humans and animals).

Domestication and relationships to evolutionary phenomena

Domestication (definition see BOX 1) of plants and animals is without doubt one of the most important developments in human history, triggering the rise of today's civilization with its complex societies, technological innovations, literacy, and vast demographic shifts (Diamond, 1999, 2002) and causing the complete transformation of most natural ecosystems on earth (Driscoll et al., 2009). Further, domestication is considered one of the largest experiments ever conducted by humans and has led to increased phenotypic variation and a great number of phenotypic characteristics not observed in the wild forebears (Darwin, 1868). These phenotypic alterations associated with domestication in plants and animals are often strikingly similar in groups which are otherwise not closely related to one another, a convergence which was first recognised by Darwin (1868) and has been summarised as 'domestication syndrome' (Ross-Ibarra et al., 2007; Wilkins et al., 2014). In mammals, the 'domestication syndrome' includes increased docility, depigmentation of skin and fur, reduction of tooth size, shortening of the rostrum, floppiness of the ears, curliness of the tail, increased fecundity, non-seasonality of oestrus cycles, hormonal changes, prolongation of juvenile behaviour, and reduction of brain size (Wilkins et al., 2014 and references therein). (NB: the term 'syndrome' in this context does not refer to a pathological condition.)

Domestication is a complex and long-term process, not only driven by humans but also by a multitude of biological, ecological, and cultural factors (Larson et al., 2014). Importantly, only wild progenitors with a set of special characteristics suitable for the life in the context of a human dominated environment are suitable for domestication, e.g., they should not have too specialised dietary preferences (Galton, 1865; Price, 1984; Diamond, 1999, 2002). For suitable species, different pathways of domestication have been proposed (Zeder, 2012; Larson & Fuller, 2014): along the 'commensal' pathway, animals are attracted to and taking advantage of elements of the human niche and subsequently develop social and/or economic bonds with humans (e.g., dog, cat). The 'prey' and 'directed' pathways, on the other hand, are initiated by humans. The species undergoing the 'prey' pathway are usually prey species (e.g., goat, sheep, cattle) which are domesticated following continuous stages of game management strategies, herd management strategies, and controlled breeding. The 'directed' pathway is an immediate and fast way of domestication, using established knowledge about previous

domestication processes. As opposed to the influence of natural and sexual selection in the wild, natural, sexual, as well as artificial selection by humans is common to all of these domestication pathways (reviewed by Driscoll et al., 2009).

Despite the different pathways that may lead to domestication, the occurrence of the 'domestication syndrome' indicates common resulting patterns among mammalian species. In breeding experiments on silver foxes (a colour phase of the red fox) that were conducted since 1959 in Siberia, D. K. Belyaev could show that selection for tameness alone leads to the expression of characteristics typical of the 'domestication syndrome' (Trut, 1999; Trut et al., 2004): already after a few generations, the foxes expressed a loss of pigmentation, floppy ears, rolled and shortened tails, shorter limbs, shortened and widened rostra, smaller brains, and earlier sexual maturity (Trut, 1999; Trut et al., 2004). This selection for tameness has been proposed to lead (via to date unknown relationships) to mild neural crest cell deficits during embryonic development which, either directly or indirectly, cause most of the characteristics of the 'domestication syndrome' (Zrzavý et al., 2009; Wilkins et al., 2014) (Fig. 1).

It has been pointed out that domestication serves as a model for the investigation of evolutionary phenomena. Clutton-Brock (1999, p.30) emphasised that "The biological process of domestication can be seen as an evolution that mimics the sequence of events in the differentiation of island races of wild animals from their mainland ancestors.". As a matter of fact, many characteristics of domesticated mammals can also be frequently observed in mammals which evolve, or have evolved, on islands (Fig. 2). These traits include alteration of body size (dwarfism/gigantism), increased size variation, shortening of the rostrum, shortening of the limbs, tameness, and reduction of brain size in some species (van der Geer et al., 2010 and references therein), although the decrease in brain size in island mammals is associated with a reduction in body size whereas this needs not to be the case in domestic mammals (Kruska, 2005; Weston & Lister, 2009)(Fig. 2). Although artificial selection is only found under domestication and not in island environments, both phenomena are characterised by the isolation from the ancestral population (van der Geer et al., 2010) (although post-divergence gene flow between wild and domestic populations is common; Larson & Burger, 2013), population bottlenecks (Clutton-Brock, 1999; Lindblad-Toh et al., 2005; van der Geer et al., 2010; Wang et al., 2013; Freedman et al., 2014), genetic drift (Clutton-Brock, 1999; van der Geer et al., 2010), the occupation of new niches (Herre & Röhrs, 1990; van der Geer

BOX 1: Definition of domestication

The intensity of the relationship between humans and animals ranges from the mere housing of wild animals to intensive breeding under human control (Clutton-Brock, 1999; Vigne, 2011; Zeder, 2012). This continuous gradient renders the definition of domestication a non-trivial matter (Larson & Burger, 2013) since it is supposed to consider forms which are associated with humans since thousands of years but breeding is not under human control, e.g., the domestic cat, and, at the same time, to exclude groups which are bred in captivity but do generally exhibit no or only few adaptations to the human dominated environment, e.g., zoo animals. Thus, definitions based on only the mastery over breeding or ownership are not sufficient (Herre & Röhrs, 1990). One of the most inclusive definition of domestication to date is therefore the colonisation of and adaptation to ecological niches generated by humans (Herre & Röhrs, 1990; Coppinger & Coppinger, 2001; Lord et al., 2013; Larson et al., 2014).

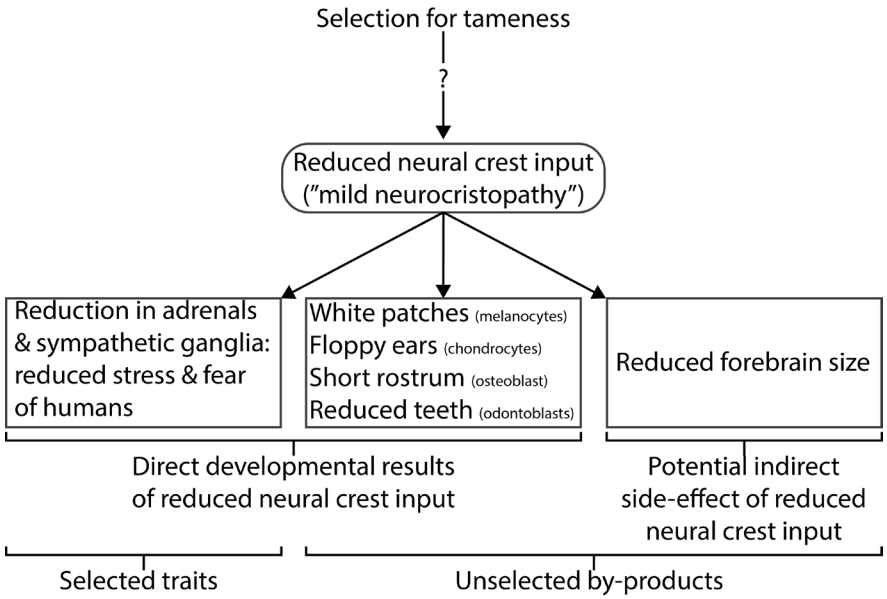

Figure 1. The neural crest hypothesis explains the occurrence of the 'domestication syndrome' in otherwise not closely related domestic animals with the initial selection for tameness. This then leads, as a cause of yet unexplained mechanisms (indicated with a question mark), to a reduced neural crest input into the sympathetic and adrenal systems. This mild neurocristopathy in turn leads, either directly or indirectly and occasionally as a by-product, to all the observed components of the 'domestication syndrome'. The arrows indicate hypothesised directions of influence on traits. Modified after Wilkins et al. (2014).

et al., 2010; Larson et al., 2014), and altered selection pressures (Fig. 2). Latter are consequences of modification/limitation of nutrition and ecological release due to decreased interspecific competition and absence of predators (Tchernov & Horwitz, 1991; Parker et al., 2010; van der Geer et al., 2010; Lomolino et al., 2012). Moreover, in both cases morphological changes tend to occur relatively fast (Lister, 1989; Millien, 2006). Because of these similarities, amongst other evidence, some island mammals have mistakenly been classified as feral domesticates. The Balearic bovid *Myotragus balearicus* has long been claimed to show clear signs of domestication, although there is to date no clear evidence for this view (Ramis & Bover, 2001). The Falkland island fox *Dusicyon australis* (Fig. 3) has been considered a feral domestic canid because of its white tail tip, rostrum, and, lower limbs, broad skull, bulbous forehead (as it is common in domestic dogs), and its tameness (Clutton-Brock, 1977). This hypothesis has been debated on the basis of divergence times which are too old as to support the hypothesis of human transportation of these animals to the island (Slater et al., 2009; Austin et al., 2013).

Domestic Dogs – the study subject

Among domestic animals, dogs are outstanding regarding their early domestication and their extreme variability. Darwin (1868) suggested that domestic dogs originate from multiple ancestral species, but it is now widely accepted that the sole ancestor of all recent domestic dogs is the wolf (*Canis lupus*) (But see Koler-Matznick, 2002). This hypothesis is supported by studies on behaviour, vocalisation, and morphology (Clutton-Brock, 1995; Grzimek, 2011 and references therein), more recently complemented by genetic studies (Vila et al., 1997; Agnarsson et al., 2010; von-Holdt et al., 2010; Freedman et al., 2014).

The timeframe as well as the geographic area(s) of dog domestication are subjects of debate (Larson & Bradley, 2014). An East Asian origin of domestic dogs has been suggested by several authors based on mitochondrial DNA (Savolainen et al., 2002; Pang et al., 2009) and genomic data (Wang et al.,

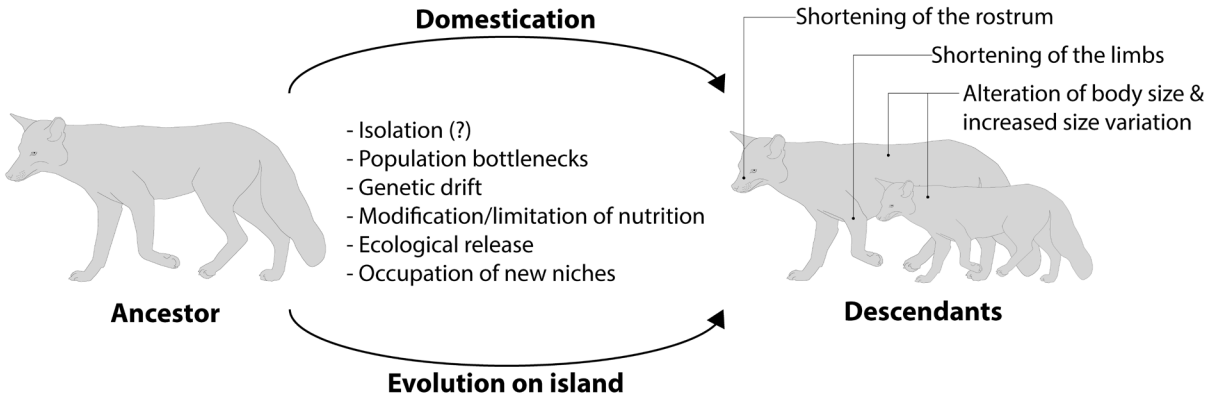

Figure 2. Schematic illustration of the similarities of patterns under domestiction and island evolution. For details and discussion see text.

2013) (But see Boyko et al., 2009). On the basis of single nucleotide polymorphisms, vonHoldt et al. (2010) provided evidence for a Middle East and European ancestry of domestic dogs. The critical importance of the middle east (fertile crescent) for the development of variation in domestic dogs has also been emphasised by the detection of certain haplotypes, which are specific for many domestic dog lineages (IGF1 which is associated with small size and FGF4 which is associated with chondrodysplasia), and which could be found exclusively in wolves of this region (Parker et al., 2010). Vila et al. (1997) and vonHoldt et al. (2010) further proposed multiple domestication events, a hypothesis which is in accordance with the great morphological diversity of early dogs (Ovodov et al., 2011; Pionnier-Capitan et al., 2011). The debate about the geographic origin of the domestic dogs is complicated by a possible total extinction of the ancestral wolf lineage today (Freedman et al., 2014). Genetic evidence has further shown that dogs were not domesticated independently in the Americas, but stemming from the Old World and passed the Bering Strait as human companions during the Late Pleistocene (Leonard et al., 2002).

Concerning the timing of the onset of dog domestication, estimates based on mitochondrial DNA range from about 15'000 years (Savolainen et al., 2002), 16'300 years (Pang et al., 2009), up to more than 100'000 years before present (Vila et al., 1997). Genomic data points to a split between wolves and domestic dogs at about 32'000 years ago (Wang et al., 2013) or 11'000 to 16'000 years ago (Freedman et al., 2014). Based on the investigation of single nucleotide polymorphisms across breeds, Lindblad-Toh et al. (2005) estimated an ancient bottleneck, and subsequently the beginning domestication process of dogs, at about 27'000 years ago. Archaeological records report about 36'500 calendar years old canid skulls from the Czech Republic, Belgium, Ukraine, and Russia with features typical for domestic dogs

Figure 3. Falkland island fox (*Dusicyon australis*) by John Gerrard Keulemans in Mivart (1890), accessed from the Biodiversity Heritage Library. Note the white hairs in tail tip, rostrum, and lower portion of the limbs.

(Germonpré et al., 2009; Germonpré et al., 2012; Germonpré et al., 2013) and 33'000 calendar year old domestic dogs from Siberia (Ovodov et al., 2011). However, the classification of these Palaeolithic findings as fully domesticated dogs has been questioned (Crockford & Kuzmin, 2012). The earliest indisputable remnants of domestic dogs are not older than the beginning of the Late Glacial (approximately 18'000 calendar years before present), ranging mostly around 14'000 to 15'000 calendar years before present (Pionnier-Capitan et al., 2011; Wayne et al., 2006 and references therein). Despite all these discrepancies, molecular and archaeological studies are not generally contradictory (see also Wayne et al., 2006). Researchers generally agree that the domestic dog is the first domesticated organism, with a presumed origin in the Late Pleistocene (Upper Palaeolithic) in hunter-gatherer societies and before the Neolithic revolution (Werneburg, 2014).

There are only few clues as to the processes by which wild wolves were transformed into domestic dogs (Grzimek, 2011), although it is not disputed that dogs were domesticated along the 'commensal' pathway (Zeuner, 1963, see above). It has been proposed that domestication started with wolves scavenging around human settlements (Coppinger & Coppinger, 2001; Grzimek, 2011). These wolves became increasingly tamer than their wild conspecifics and were accepted by humans as garbage collectors, 'watch dogs', and perhaps occasional food item (Coppinger & Coppinger, 2001; Grzimek, 2011; Lindsay, 2013). However, this model of 'self-domestication' cannot explain the origin of the close social bond that is typical for historical and extant human-dog partnerships (Grzimek, 2011). It has therefore been proposed that the social partnership might be based on deliberate capturing, adopting, and keeping of young wolves, a practice which is still widespread among contemporary hunter-gatherer societies, but which in turn does not account for the still great nervousness and fearfulness of adult wolves in captivity (Grzimek, 2011). Both of these models together might serve as a good approach to an understanding of initial dog domestication.

Subsequently to the initial self-domestication process, isolation, genetic drift, natural selection, and unconscious artificial selection by humans (probably for increased tameness) differentiated the scavenging wolves from wild wolf populations (Driscoll et al., 2009). Since then, artificial selection (unconscious and later conscious) has led to an ever increasing morphological as well as behavioural variability of domestic dogs. Different types of dogs could already be distinguished in the Pre-Pottery Neolithic of the Near East (about 8670 years before present) and in Ancient Egypt (prior to 5000 years before present), but a great increase in the number of different types took place during Medieval times and the Renaissance (Zeuner, 1963; Galibert et al., 2011). Domestic dogs became increasingly adapted to fulfil various tasks and many specialised groups are discernible today, e.g., solitary or pack hunting dogs with or without the lead of humans, bulldogs specialised for fighting or holding down prey or slaughter cattle, livestock guardian dogs, cattle dogs, herding dogs, yard dogs, toy dogs, and sledge dogs (Coppinger & Schneider, 1995; Krämer, 2013). Despite the long stading differentiation of functional groups of domestic dogs, modern breeds are relatively young. Only in the course of the 19th century, modern breeding practices with strict aesthetic requirements and closed bloodlines for newly defined distinct breeds emerged. These practices have led to the domestic dog being the most variable mammalian population known, with one of the largest and heaviest breeds, the St. Bernard, being more than 3 times as tall and more than 30 times as heavy as the smallest breed, the Chihuahua (see title page image).

The histories of domestic dog and humans are interwoven to a great degree, leading to parallel evolution of some genes in both species (most apparent in genes for digestion,

metabolism, neurological processes, and cancer; Wang et al., 2013). Moreover, many genes with key roles in starch digestion and fat metabolism have been found in dogs, indicating specific adaptations to their novel environment during early domestication (Axelsson et al., 2013). Further, the same genes cause similar diseases in domestic dogs and man, making dogs a model species (Karlsson & Lindblad-Toh, 2008; Parker et al., 2010). Domesticated dogs have further been subject to a great number of studies concerned with the mechanisms generating their peculiar characteristics. A lot is known for example about the genetics behind the generation of small body size (Sutter et al., 2007), different coat colours and textures (Schmutz & Berryere, 2007; Cadieu et al., 2009), differences in relative limb length (Parker et al., 2009), and differences in skull shape (Fondon & Garner, 2004; Schoenebeck & Ostrander, 2013). To conclude, the tremendous disparity and relatively well known biology of domestic dogs renders them an ideal study system for addressing questions on patterns of genetic and phenotypic evolution.

In the context of island evolution, the investigation of domestic dogs is probably not ideal because members of Carnivora are generally rare on islands; reasons for this might be their poor abilities to disperse overseas, their dependency on prey, and their often solitary lifestyle (van der Geer et al., 2010). Fossil canids which were endemic on islands are the Sardinian dogs *Cynotherium* sp. (Middle Pleistocene), *Cynotherium malatestai* (?late Early Pleistocene and/or Early Middle Pleistocene), and *Cynotherium sardous* (Late Pleistocene) of Sardinia and Corsica (Malatesta, 1970; Lyras et al., 2006; Madurell-Malapeira et al., 2015), the Java dog *Megacyon merriami* (late Early Pleistocene) and the Trinil dog *Mececyon trinilensis* (Middle Pleistocene) from Java (Schütt, 1973a, 1973b), the Falkland fox *Dusicyon australis* (Slater et al., 2009; Austin et al., 2013), and the Japanese (Honshu) wolf *Canis lupus hodophilax* and the Hokkaido wolf *Canis lupus hattai* (Endo et al., 1997; Ishiguro et al., 2009; Walker, 2009; Ishiguro et al., 2010). Latter three went extinct only recently trough human intrusion. Only one endemic island canid is extant, the island grey fox *Urocyon littoralis* from the Channel islands in California (Wayne et al., 1991; Moore & Collins, 1995). Whilst some island canids are remarkably smaller than their mainland ancestor, some others did not change body size or got even larger (Lyras et al., 2010). Apart from changes in body size, recent and extinct island canids reveal anatomical features of their skeleton, teeth, fur, and behaviour, which parallel anatomical peculiarities that can also be found in some domestic dog breeds (see above), although changes of anatomical features in island canids do not follow a consistent pattern (van der Geer et al., 2010). For example, first, missing premolars in the lower jaw of a *C. sardosus*, without any trace of an alveolus, have been reported (Malatesta, 1970; Lyras et al., 2006). These findings might point to an evolutionary trend toward a reduction of the premolars (Lyras et al., 2006), as seen in many domestic dogs (Butković et al., 2001). Second, in comparison its mainland ancestor *U. cinereoargenteus*, *U. littoralis* exhibits relatively wide nasal bones and relative short legs as well as a general lack of fear of humans (Moore & Collins, 1995). Third, as already described above, *Dusicyon australis* had a relative wide palate, expanded frontal bones, a bulbous forehead, was docile, and had compacted teeth in the premolar region (Clutton-Brock, 1977).

Bones and teeth – the study systems

Due to extensive mineralisation, bones and teeth resist decay to a great degree and are therefore crucial subjects of zoological, palaeontological, and archaeological research on mammals (and also other vertebrates). They permit the investigation of evolutionary changes of the anatomy (e.g., Hall, 2005; Ungar, 2010), phylogenetic relatedness of clades (e.g., Smith, 1994a; Hofreiter et al., 2001),

life history and metabolism (e.g., Köhler & Moyà-Solà, 2009; Marín-Moratalla et al., 2013; Kolb et al., 2015), dietary habits (e.g., (Bocherens et al., 1994), pathological conditions of individuals (e.g., Siegel, 1976), and ontogenetic patterns (e.g., Sánchez-Villagra, 2012), depending on the level of integration which is investigated (Francillon-Vieillot et al., 1990). Especially the examination of the role of ontogeny in the generation of disparity in adult morphology provides a rich field of investigation (evolutionary developmental biology; Gould, 1977; Hall, 2012).

During postnatal ontogeny, bones grow in size either intramembranously (membrane bones and at sutural margins of cranial bones), subperiosteally (along the shaft of long bones), or by endochondral replacement (e.g., at the growth plates of vertebrae and long bones) (Hall, 2005). The state of deciduous tooth eruption at birth differs greatly among mammalian species but the eruption of the permanent dentition, on the other hand, is generally confined to the postnatal period (Anders et al., 2011 and references therein). Postnatal ontogeny is therefore a crucial period in the mammalian life cycle and provides valuable information on the generation of disparity. The importance of postnatal growth on the generation of interbreed disparity in domestic dogs could further be emphasised in an ongoing study which I am conducting as a side project to this thesis in collaboration with I. Werneburg (BOX 2). Furthermore, postnatal stages are more readily available in museum collections otherwise than embryos, and direct comparisons with fossils are in many cases possible (Geiger et al., 2013).

Postnatal markers of growth on the macro-anatomical level of integration are fusion of growth plates in the postcranial skeleton, unification of primary ossification centres (e.g., pelvis, sutures of the skull), and eruption of teeth (Buikstra & Ubelaker, 1994). These markers of growth are handy tools for the investigation of postnatal growth, especially cessation of growth, because growth plates and sutures serve as major sites of bone expansion and their closure is an indicator for cessation of growth at that site. The markers are moreover readily investigable in fossil as well as in recent skeletal material. Further, cessation of skeletal and dental growth has repeatedly brought in conjunction with life history events (Shigehara, 1980; Smith, 1992), making these markers to an interesting tool for the investigation of evolutionary patterns.

Study aims

The main aim of this PhD thesis is to elucidate whether heterochronic changes of patterns of postnatal growth (tooth eruption, suture closure, growth plate closure, ontogenetic cranial shape change) occur throughout the domestication process of dogs, also in association with life history variables (Fig. 5). However, as a first approach, some aspects of these issues were examined across a comprehensive sample of wild mammalian species.

The numerous growth plates and sutures do generally not fuse simultaneously and also the teeth do not erupt all at the same time, but in a sequence. Sequences and timing of fusion and eruption have been investigated to some extent in several mammalian species and in some domestic animals. However, so far most studies have been restricted to single species or few, relatively closely related species (for references on growth plates see *Chapter 1)* and only few works treat evolutionary trends within higher order clades, e.g., sequences of epiphyseal union in ‘ungulates’ (Todd & Todd, 1938) and primates (Hofer et al., 1956) or sequences of tooth eruption in insectivores and carnivorans (Slaughter et al., 1974) and primates (Smith, 1994). Relatively recently, more efforts have been made, also in our group, to provide such syntheses for tooth eruption sequences in cervids (Veitschegger & Sánchez-Villagra, submitted) and suture closure sequences in hystricognath and sciurid rodents (Wilson & Sánchez-Villagra, 2009; Wilson, 2014), deer (Sánchez-Villagra,

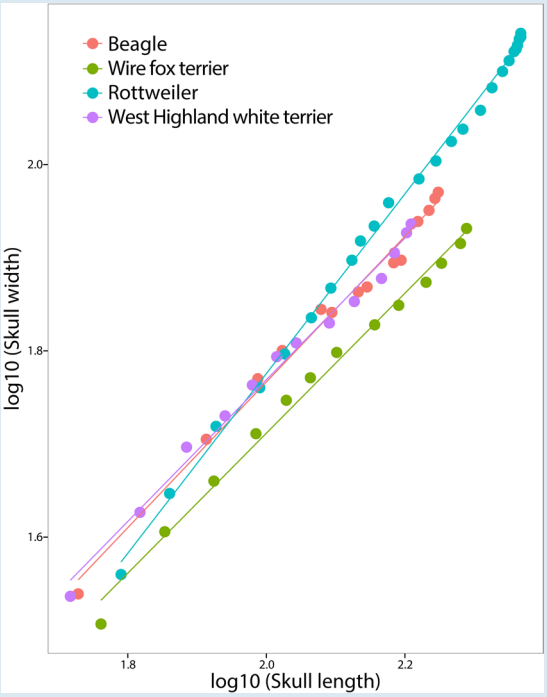

Figure 4. Postnatal growth trajectories of skull length and width in different domestic dog breeds (Böhme, 1994; Baumbach, 1999; Kaiser, 2003; Kreher, 2005; Schubert, 2011). This comparison illustrates similarities in trajectories of the beagle, wire fox terrier and West Highland white terrier, whereas the slope of the trajectory of the Rottweiler is significantly different from the other breeds.

Table 1. Linear measurements (mm) and one index (length-width index, as calculated from head length divided by head width) on the heads and limbs of growth series of Beagle (Böhme, 1994), Bernese mountain dog (Kaiser, 2003), Rottweiler (Baumbach, 1999), West Highland white terrier (Schubert, 2011), and wire fox terrier (Kreher, 2005) and associated standard deviations (sd) among the values of neonates (W0) and adults (WA).

Measurement	sd (W0)	sd (WA)
Head width	2.61	22.66
Head length	4.22	31.13
Length-width-index	0.11	0.20
Upper arm length	3.21	33.73
Forearm length	3.84	48.36
Tight length	4.65	49.85
Lower leg length	3.47	41.03

BOX 2: Importance of post-natal growth for the generation of interbreed disparity

Breed specific characteristics of skull and limb dimensions and proportions can already be recognised in newborn puppies (Starck, 1962; Rosenberg, 1966; Wayne, 1986). As gestation length does neither scale with body size nor mass in domestic animals in general (Clauss et al., 2014) and domestic dogs in particular (Kirkwood, 1985), it has been suggested that the growth rate during prenatal development is variable among the breeds (Wayne, 1986; Clauss et al., 2014). During postnatal life, on the other hand, the growth rates have been reported to be relatively similar among domestic dog breeds (Rosenberg, 1966; Wayne, 1986). To further investigate growth rate among domestic dog breeds and the extent of contribution of post-natal ontogeny to adult phenotypic disparity, we conducted a literature review, analysing data on post-natal growth series of different breeds (Geiger & Werneburg, in prep.). First, linear skull measurements were compared among newborn and fully grown adults of four morphological variable breeds, the beagle (Böhme, 1994), Bernese mountain dog (Kaiser, 2003), Rottweiler (Baumbach, 1999), West Highland white terrier (Schubert, 2011), and wire fox terrier (Kreher, 2005). Second, am ANCOVA was used to recognise differences of regression slopes between the trajectories of head length versus head width of these breeds (the Bernese mountain dog could not be used in the second analysis due to non-availability of raw data). Table 1 shows the measurements as well as the length-width index and associated standard deviations among newborns and adults. It is evident that the differences among the adults of the different breeds are generally greater than the differences among the newborns. If the growth in width of skull is compared to growth in length, the regression slopes of the beagle , wire fox terrier, and West Highland white terrier are similar to each other, confirming previous results about similar growth rates during postnatal development. However, the slope of the Rottweiler is significantly different to all the other breeds ($p < 0.0001$), indicating differences of the postnatal growth rate (Fig. 4). Although bivariate comparisons of skull measurements do not accurately reflect the tremendous variability of skull shapes in domestic dogs, these results emphasise the importance of postnatal growth on the generation of breed-specific morphological disparity.

2010), carnivorans (Goswami et al., 2013), and the entire mammalian clade, including marsupials and placentals (Rager et al., 2014). *Chapter 1* of my thesis expands for the first time and in a comprehensive way this series of studies to the postcranium by investigating the sequences of growth plate closure across mammals. In particular, it was tested, whether the growth plate closure sequence is the same across Mammalia, as hypothesised previously (Stevenson, 1924) and if heterochronic shifts, if present, characterise certain clades or locomotor groups. Special emphasis was made on the investigation of the marsupial-placental dichotomy and heterochronies in the growth plate closure sequences associated with it.

Chapter 2 is concerned with patterns of growth plate closure, dental eruption, and sexual maturity. It aims at testing an old standing hypothesis about the relationships of the age at attainment of skeletal, dental, and sexual maturity in domestic dogs and wolves. It has been proposed that this sequence is altered in dogs because of changed environmental conditions during domestication (Tchernov & Horwitz, 1991). Specifically, wolves are thought to attain first dental maturity, second skeletal maturity, and third sexual maturity. In domestic dogs this sequence is supposed to be altered so that sexual maturity is attained before dental and skeletal maturity (Shigehara, 1980). Since this hypothesis has so far been

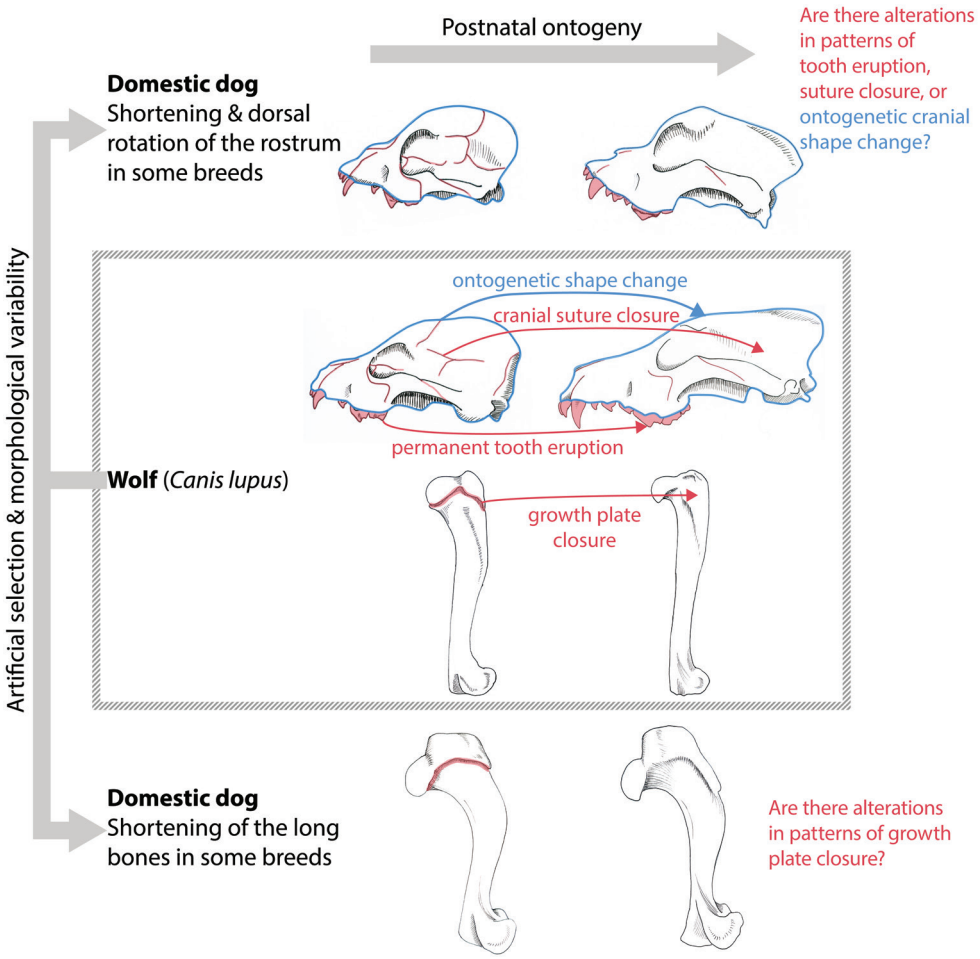

Figure 5. Schematic illustration of the smain aims of this PhD thesis. Artificial selection during domestication has led to great morphological variability in domestic dogs (e.g., shortening of long bones and alterations of skull shape, including relative shortening of the rostrum). This thesis aims at elucidating whether patterns of postnatal growth, i.e., tooth eruption, suture closure, growth plate closure, ontogenetic cranial shape change, are altered in domestic dogs as compared to the wolf ancestor and whether these patterns vary among domestic dogs exhibiting different morphologies.

based on only a few literature records, we aimed at testing this hypothesis by incorporating original data on the timing of tooth eruption and growth plate closure in different and distinct breeds of dogs.

Chapter 3 treats patterns of suture closure in different breeds of domestic dogs. We investigated whether domestic dogs with a short face and dorsally rotated rostrum (bulldog-type breeds) exhibit comparable suture and synchondroses closure patterns as humans with a similarly reduced face as a cause of certain syndromes (Achondroplasia, Crouzon, Apert, and Pfeiffer syndromes) which are associated with premature suture and synchondrosis closure.

Chapter 4 takes further a long standing debate on developmental heterochrony of skull shape during the domestication process of dogs. It has been discussed whether the peculiar skull shapes observed in many domestic animals, e.g., short and broad rostrum, are the result of juvenilisation (paedomorphosis), i.e., processes leading to the retention of juvenile wolf features in adult domestic dogs.

The document appended to this thesis represents an additional manuscript on an area which is methodologically related with the main aspects of *Chapter 4* of this thesis. In this project (co-supervision of a MSc-project in our group), ontogenetic trajectories of cranial and mandibular shapes in cave bears (*Ursus spelaeus*) were compared to ontogenetic trajectories of extant bear species (*U. arctos, U. maritimus*, *U. americanus*) using a geometric morphometrics approach.

Thesis outline

This cumulative thesis presents all chapters subsequent to this one either as fully-formatted article as published in the respective journal (*Chapter 1*) or in manuscript form (*Chapters 2* to *4*, *Appendix*). Authors, publication details, and author and co-author contributions are provided at the beginning of each chapter. Supplementary material is provided at the end of each chapter.

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CHAPTER 1

Heterochrony and post-natal growth in mammals – an examination of growth plates in limbs

This work was done during the first year of my PhD. AF and I collected the data. I conducted the analyses with the help of DK. I wrote the manuscript and all authors then participated in discussing and drafting the final manuscript.

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Heterochrony and post-natal growth in mammals – an examination of growth plates in limbs

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Abstract

Mammals display a broad spectrum of limb specializations coupled with different locomotor strategies and habitat occupation. This anatomical diversity reflects different patterns of development and growth, including the timing of epiphyseal growth plate closure in the long bones of the skeleton. We investigated the sequence of union in 15 growth plates in the limbs of about 400 specimens, representing 58 mammalian species: 34 placentals, 23 marsupials and one monotreme. We found a common general pattern of growth plate closure sequence, but one that is universal neither between species nor in higher-order taxa. Locomotor habitat has no detectable correlation with the growth plate closure sequence, but observed patterns indicate that growth plate closure sequence is determined more strongly through phylogenetic factors. For example, the girdle elements (acetabulum and coracoid process) always ossify first in marsupials, whereas the distal humerus is fused before the girdle elements in some placentals. We also found that heterochronic shifts (changes in timing) in the growth plate closure sequence of marsupials occur with a higher rate than in placentals. This presents a contrast with the more limited variation in timing and morphospace occupation typical for marsupial development. Moreover, unlike placentals, marsupials maintain many epiphyses separated throughout life. However, as complete union of all epiphyseal growth plates is recorded in monotremes, the marsupial condition might represent the derived state.

Introduction

Mammals display a broad spectrum of limb morphology coupled with different locomotor strategies and habitat occupation (Polly, 2007). Structural differences in form between species often reflect functional specializations (Farnum, 2007; Polly, 2007). The individual limb phenotype is formed during ontogeny, and as selection can act on any level of biological organization and at any time during ontogeny, the possibilities for creating different limb morphologies are numerous (Farnum, 2007). One important aspect in limb evolution that leads to interspecific structural difference is the change of timing (i.e. heterochrony) of individual events in skeletal ontogeny (Richardson *et al.*, 2009). Heterochronic shifts were found to be responsible for disparity in body proportions not only in prenatal development, but also during post-natal growth (Raff & Wray, 1989; Farnum, 2007). For example, it has been shown that allometric growth and growth heterochrony in limbs of different primates lead to proportion differences that are potential adaptations to different locomotor behaviours (Shea, 1993).

One aspect of post-natal long bone growth includes elongation by increment at their cartilaginous ends (Hall, 2005; Farnum, 2007). Mammals build a secondary ossification centre within this cartilage, and longitudinal growth is restricted to the cartilaginous growth plate between the bony metaphysis and the secondary ossification centre (Hall, 2005; Farnum, 2007). This cartilaginous growth plate grows by division and vol-

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ume increase in the cartilage cells before it is replaced by bone (endochondral ossification) (Hall, 2005; Farnum, 2007). When the finite proliferative capacity of the chondrocytes is exhausted, longitudinal bone growth ceases and bony union occurs (Parfitt, 2002; Nilsson & Baron, 2004; Hall, 2005). The growth plates of all long bones of a skeleton fuse at different times, resulting in a sequence of growth plate union. Variation in this sequence among different mammalian clades remains understudied, mainly due to a widespread view that post-cranial growth plate closure is evolutionary conserved across Mammalia (King *et al.*, 2001). Stevenson (1924, p. 89) stated that the particular sequence of epiphyseal union reported in his study 'may be considered a general mammalian trait'. Further evidence for this hypothesis was then provided by Dawson (1925), Todd (1930a,b), Koch (1935) and Todd & Todd (1938). Contrarily, other analyses have demonstrated a variation in growth plate closure sequences among mammalian species (Washburn, 1946; Shigehara, 1980).

To summarize, it is not clear whether there is disparity in epiphyseal growth plate closure sequence and whether heterochronic shifts – if they exist – influence limb proportions and hence functional adaptations. In our study, we approached this problem by asking to what extent the epiphyseal growth plate closure sequence is influenced by intrinsic (phylogenetic relationships) (Weisbecker *et al.*, 2008) and extrinsic (different biomechanical loadings through different locomotor habits) (Carter *et al.*, 1991) factors. This is the first study that investigates this issue in a wide spectrum of species that are representatives of all major mammalian clades.

We expect that influences of phylogeny may cause the most heterochronic changes in higher-order taxa because of their greater amount of morphological and physiological discrepancies. Examples are the marsupial–placental dichotomy (Sánchez-Villagra, 2002; Weisbecker *et al.*, 2008), the 'Atlantogenata'–Boreoeutheria dichotomy and the Euarchontoglires–Laurasiatheria dichotomy (Asher *et al.*, 2009). The functional aspects that may lead to heterochronic shifts in growth plate closure sequence include the function of single joints adjacent to the growth plates. This aspect is important regarding mechanical loadings. We therefore expect that the growth plate closure sequences of members of one locomotor group are more similar to one another than they are to sequences of taxa from other locomotor groups.

In this study, we investigated the sequence of growth plate closure in mammals. First, we evaluated whether growth plate closure patterns are conserved among the studied mammalian species and display the patterns originally found by Stevenson (1924). Second, we examined whether heterochronic shifts and peculiarities in growth plate closure sequence characterize major mammalian clades. Third, we investigated whether adaptations to different locomotor styles include heterochronic shifts in growth plate closure sequences. Further, we studied growth plate closure in relation to relative age.

Materials and methods

Specimens

We examined a cross-sectional ontogenetic sample comprising 395 skeletons representing 34 genera and 58 species: 34 placentals, 23 marsupials and 1 monotreme (Table 1, Table S1). A list of the investigated specimens is available from the Dryad Digital Repository. In nine genera, more than one species per genus was analysed (Fig. 1, Table S1). The maximum number of species included in one genus is seven (*Macropus* spp.). On average, genera included 11.6 specimens (max. 29 specimens; min. four specimens) (Table 1). Taxa were selected based on the availability of specimens representing multiple ontogenetic stages and to represent ecological and phylogenetic breadth (Fig. 1). Captive and wild-caught animals were sampled, and both sexes were used. Domesticated species (e.g. *Felis catus*, *Equus caballus caballus/Equus ferus caballus*) and skeletons exhibiting pathologies were excluded from the study. Also excluded were specimens with all growth plates open or all growth plates closed.

Data were collected from specimens at the following institutions: AIM, Anthropologisches Institut und Museum der Universität Zürich; MNHN, Muséum national d'Histoire naturelle, Paris; NHM, The Natural History Museum, London; NMB, Naturhistorisches Museum Basel; NMBE, Naturhistorisches Museum Bern; NRM, Naturhistoriska Riksmuseet, Stockholm; PIMUZ, Paläontologisches Institut und Museum der Universität Zürich; SZ, Zoologische Schausammlung der Universität Tübingen; UMZC, University Museum of Zoology, Cambridge University; ZMM_MAM, Museum für Naturkunde, Berlin; ZMUZH, Zoologisches Museum der Universität Zürich; and ZSM, Zoologische Staatssammlung München.

Data collection

The closure states in 15 growth plates in the fore- and hindlimbs were scored: coracoid process of the scapula; proximal humerus; distal humerus; proximal ulna; distal ulna; proximal radius; distal radius; acetabulum of the pelvis; head of the femur; greater trochanter of the femur; distal femur; proximal tibia; distal tibia; proximal fibula; and distal fibula. In *Capreolus capreolus* and *Equus* spp., only 13 and 12 growth plate sutures, respectively, could be scored, because of an extensive reduction in the fibula in both genera and absence of the distal ulna in *Equus* spp. (Schmid, 1972). Only specimens with a complete record of closure stages in all growth plates were used for the analyses. Growth

Table 1 Genus and species names (Wilson & Reeder, 2005), taxonomic designation (Wilson & Reeder, 2005) and colloquial names (Nowak, 1999; Wilson & Reeder, 2005); number of specimens per genus/species examined (*n*) and locomotor category (Nowak, 1999) of investigated taxa.

Taxa	Colloquial name	*n*	Locomotor category
Monotremata			
Tachyglossus aculeatus	Short-nosed echidna	7	Terrestrial
Marsupialia			
Didelphimorphia			
Didelphis spp.	Large American opossum	11	Terrestrial
Diprotodontia			
Dorcopsis muelleri	Brown dorcopsis	6	Bipedal saltatorial
Macropus spp.	Wallaby/wallaroo /kangaroo	22	Bipedal saltatorial
Phascolarctos cinereus	Koala	4	Arboreal
Dasyuromorphia			
Thylacinus cynocephalus	Thylacine/Tasmanian tiger	9	Cursorial
Sarcophilus harrisii	Tasmanian devil	8	Terrestrial
Dasyurus spp.	Native 'cat'/tiger 'cat'/quoll	10	Terrestrial /scansorial
Peramelemorphia			
Perameles spp.	Long-nosed bandicoot	6	Terrestrial
Isoodon spp.	Short-nosed bandicoot	4	Terrestrial
Placentalia			
Cingulata			
Dasypus spp.	Long-nosed armadillo	5	Semi-fossorial
Pilosa			
Myrmecophaga tridactyla	Giant anteater	5	Terrestrial
Hyracoidea			
Heterohyrax brucei	Gray hyrax/yellow-spotted hyrax	7	Scansorial
Procavia capensis	Rock dassie/rock hyrax	13	Scansorial
Tubulidentata			
Orycteropus afer	Aardvark	6	Semi-fossorial
Afrosoricida			
Tenrec ecaudatus	Tenrec	6	Terrestrial
Erinaceomorpha			
Erinaceus europaeus	West European hedgehog	21	Terrestrial
Artiodactyla			
Capreolus capreolus	European roe deer	14	Cursorial
Perissodactyla			
Equus spp.	Horse, zebra, and ass	12	Cursorial
Primates			
Perodicticus potto	Potto	16	Arboreal
Chlorocebus aethiops	Grivet	15	Scansorial
Macaca mulatta	Rhesus monkey	25	Scansorial
Papio spp.	Baboon	19	Terrestrial
Pan troglodytes	Common chimpanzee	5	Scansorial
Saimiri spp.	Squirrel monkey	11	Arboreal
Rodentia			
Marmota marmota	Alpine marmot	9	Semi-fossorial
Castor fiber	Eurasian beaver	21	Semi-aquatic
Myocastor coypus	Nutria/coypu	11	Semi-aquatic
Hydrochoeris hydrochaeris	Capybara	5	Semi-aquatic
Dasyprocta leporina	Red-rumped agouti	7	Cursorial
Lagomorpha			
Lepus europaeus	European hare	20	Cursorial
Carnivora			
Phoca vitulina	Harbor seal /common seal	29	Aquatic
Ursus arctos	Brown bear	15	Terrestrial
Felis silvestris	Wildcat	11	Scansorial

plates from the left and the right sides of the body were used.

Growth plates were scored as either open or closed. A suture was considered as closed if the outer surface of the growth plate suture was partly or completely obliterated by bone. Epiphyseal union begins in the central parts of the growth plate and subsequently extends to the outer surface (Todd, 1930a). Therefore, obliteration of the outer surface of the growth plate suture signifies the latest possible point in time during ontogeny when longitudinal growth stops. All closure stages of every investigated specimen are available from the Dryad Digital Repository. Additionally, well-documented and comprehensive literature data were considered in the analysis.

Tooth wear and locomotor groups

Generalized individual dental age stages (IDAS; Anders *et al.*, 2011) were used to document the degree of tooth eruption and wear and hence individual age class: IDAS 1, infant (period from birth to the complete eruption and beginning wear of the first molar); IDAS 2, juvenile (covers the time until the entire permanent dentition is fully erupted); IDAS 3, adult (covers the time until the loss of the inner profile in the first molar, that is, no enamel, except at the rim, is left on the occlusal surface); IDAS 4, late adult (covers the time until the complete loss of the inner profile in the second molar); IDAS 5, senile (the occlusal surface of the entire dentition is heavily worn, with the loss of functional structures and breakdown of the dentition). *Phoca vituli-*

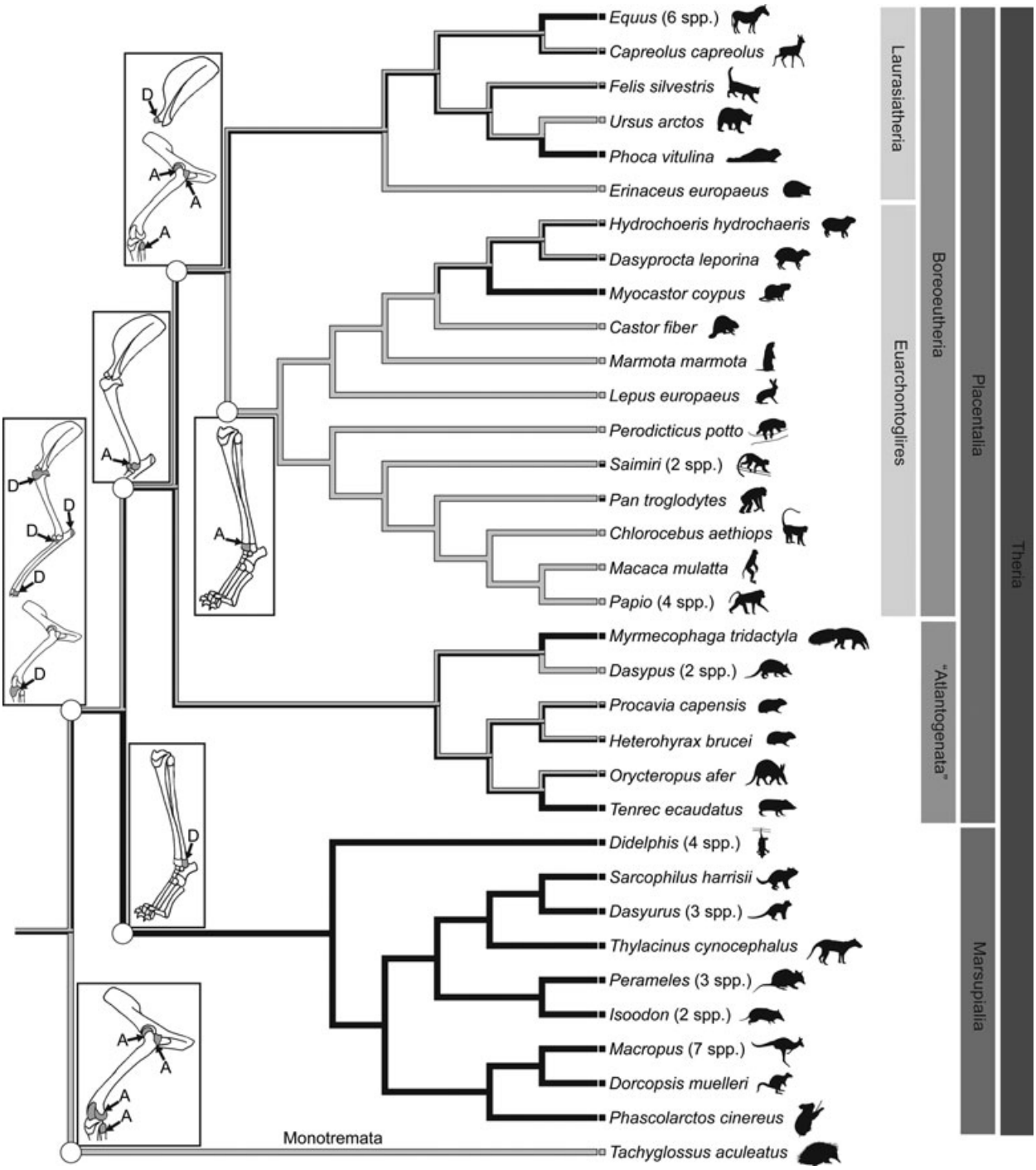

Fig. 1 Compound phylogeny featuring species and genera investigated (for references see text): 24 placentals, nine marsupials and one monotreme as outgroup. Taxonomic names of groups are based on Asher & Helgen (2010). Different shades of branches illustrate traced character history (most parsimonious solution) of the anatomical region that is fused first. Grey: an element of the elbow is fused first, black: an element of the girdles is fused first (for categorization of anatomical regions, see Fig. 2). Polymorphic branches (shown with both shades) indicate terminal taxa in which both conditions can be found. Squares at the tips of the branches indicate the conditions that were found in the investigated specimens. Boxes illustrate heterochronic changes in growth plate fusion that were detected using Parsimov-based genetic inference (PGi) (Harrison & Larsson, 2008). Branch lengths do not reflect those in the analysis. A: acceleration; D: delay.

na was excluded from the tooth wear analysis because of the homodont post-canines. Data about the IDAS of the investigated specimens are available from the Dryad Digital Repository. We acknowledge the biases introduced by the fact that the species studied exhibit different dental replacement patterns (van Nievelt & Smith, 2005) and that the wear stages in the similar loci are not fully comparable. However, these wear stages provide an age estimation that serves to examine the general pattern we wish to investigate.

Species were assigned to locomotor categories, and almost all investigated species of a genus perform the same mode of locomotion (Nowak, 1999) (Table 2). The exception is *Dasyurus*, which was categorized as terrestrial/scansorial because *Dasyurus maculatus* is scansorial, whereas the other species of the genus analysed are terrestrial. *Thylacinus cynocephalus* and *Dasyprocta leporina* are less cursorial than *Equus* spp. and *Capreolus* spp., although they are classified in the same group following the literature (Nowak, 1999). *Lepus europaeus* is a quadrupedal saltator. This is a specialization of the cursorial lifestyle and different from the bipedal saltatorial locomotion of *Macropus* spp. (Polly, 2007). *Dorcopsis* spp. shows fewer specializations associated with bipedal saltatorial locomotion than *Macropus* spp., although they are classified under the same category. Even though in the same locomotor group of semi-fossoriality, *Marmota marmota* exhibits only a few structural specializations for digging, whereas *Orycteropus afer* and *Dasypus* spp. are highly specialized. However, all three taxa are scratch diggers (Polly, 2007).

Table 2 Locomotor categories used in the analyses and their definitions (parts of definitions are modified from Samuels & Van Valkenburgh, 2008).

Locomotor category	Definition
Terrestrial	Ground dwelling. Perhaps able to run, swim, dig and climb, but none of these activities is performed extensively
Scansorial	Good capability to climb. Foraging and escape on the ground as well as in trees and/or on outcrops
Arboreal	Living in trees. Grasping capability. The ground is frequented only occasionally. Foraging and escape mostly off the ground
Cursorial	Capability to reach high speed, for example, for escape
Semi-aquatic	Swim for dispersal, escape, or foraging, but maintaining the ability to disperse across or acquire food on land
Aquatic	Swim for dispersal, escape, or forage. Do not usually disperse across or acquire food on land
Semi-fossorial	Digging to build burrows for shelter, but no foraging underground
Bipedal saltatorial	Capable of jumping behaviour characterized by simultaneous use of the hindlimbs and the tail

Phylogenetic framework

For heterochrony analyses, we used the phylogenetic tree of Meredith *et al.* (2011) for the major clades. The phylogenetic position of xenarthrans and thus the monophyly of 'Atlantogenata' are subjects of controversy. Meredith *et al.* (2011) supported the monophyly of 'Atlantogenata', although the position of the placental root is still debated and it is suggested that either afrotherians, xenarthrans, or boreoeutherians are basal to the placental mammalian tree (Murphy *et al.*, 2007; Nishihara *et al.*, 2009; O'Leary *et al.*, 2013). Therefore, 'Atlantogenata' is referred here under quotation marks. The position of Perissodactyla within Laurasiatheria is also under debate, and it is suggested that Perissodactyla is either the sister group of Artiodactyla or Ferae (Carnivora and Pholidota) (Asher *et al.*, 2009). Hallström *et al.* (2011) suggested that the genome evolution of Laurasiatheria was network-like, resulting in a hard polytomy between Perissodactyla, Artiodactyla, Carnivora, and Chiroptera. In this study, we used the phylogenetic relationships suggested by Asher (2007), Prasad *et al.* (2008), Meredith *et al.* (2011) and Zhou *et al.* (2012), which considered Artiodactyla as being the sister group of Perissodactyla. Within Rodentia and Caviomorpha, the studies by Blanga-Kanfi *et al.* (2009) and Fabre *et al.* (2012), respectively, were used. The relationships within Cercopithecidae are according to Perelman *et al.* (2011). *Thylacinus cynocephalus* was positioned basal to the Dasyuromorphia as proposed by Miller *et al.* (2009). The resulting, compound phylogenetic tree is shown in Fig. 1. To estimate the evolutionary rates of the growth plate closure sequences and to correct the adjusted closure scores for influences of different degrees of phylogenetic relatedness of the terminal taxa, we used the timetree provided by dos Reis *et al.* (2012), which is based on 36 nuclear and 274 mitochondrial genomes, combined with fossil calibrations. In those cases, where the terminal taxa from dos Reis *et al.* (2012) were not congruent with our terminal taxa, the branch length estimates of the closest relative were used. In cases where dos Reis *et al.* (2012) provided estimates for a major clade, but not for taxa within that clade, we used the known divergence time of that major clade and evenly distributed the branch lengths between the nodes of unknown age within that major clade.

Growth plate closure pattern conservation and variation

The sequence of growth plate closure was determined for every species or genus, when appropriate. The stage of closure of each growth plate ('open' = 1 or 'close' = 0) was summed up per species or genus. Following the assumption that growth plates that close early in life would be found to be closed in many specimens within an ontogenetic series, we assumed that

high numbers resulting from the sums for a particular growth plate indicate early closure of that growth plate.

To compare the sequences and to examine the rank variation in a particular closure event, each rank was adjusted following Laurin & Germain (2011):

$$r_s = (r - r_{\min})/(r_{\max} - r_{\min}),$$

where r_s is the adjusted value of a rank, r is the absolute rank of a given closure event, $r_{\min}$ is the lowest observed number of r ($r_{\min} = 1$ in this study), and $r_{\max}$ is the highest observed number of ranks for this taxon. This normalization was used because it diminishes the impact of differing numbers of ranks and hence different resolution (Koyabu *et al.*, 2011). One-way ANOVA and Tukey's b *post hoc* tests were performed to compare the means of adjusted ranks per growth plate within marsupials and placentals, 'Atlantogenata' and Boreoeutheria, as well as Laurasiatheria and Euarchontoglires separately. Additionally, one-way ANOVA was performed to investigate the sequences between major groups. This analysis was performed a second time using adjusted closure scores that were previously corrected for influences of different degrees of phylogenetic relatedness of the terminal taxa. This adjustment was computed as described in Meslin *et al.* (2012). We calculated the 78% confidence intervals of the inferred ancestral value of the common ancestor of marsupials and of the common ancestor of placentals using the PDAP:PDTREE package, version 1.16 (Midford *et al.*, 2011), and Mesquite, version 2.75, respectively (Maddison & Maddison, 2011). Using this calculation, it was possible to assess whether the differences in the ancestral values of each clade are statistically significant.

Adjusted sequences were compared for higher-order clades and locomotor groups using Kendall's coefficient of concordance (Kendall's W), a nonparametric algorithm that is used to measure the agreement between several sequences (Field, 2005). Values for W can vary between 0 (no agreement) and 1 (perfect agreement). This analysis was repeated four times with sequence data from all 15 growth plates, with data from anatomical regions (Fig. 2), and separately 'pressure epiphyses' (positioned perpendicular to the bone axis and compressed by the gravitational force and reaction force of the adjacent diaphysis) and 'traction epiphyses' (provide attachment for tendons and muscles and are subject to lateral stresses and tension) (Parsons, 1904; Serrano *et al.*, 2011) (Fig. 2).

The human closure sequence found by Stevenson (1924) was considered to be a universal pattern in mammals (Todd, 1930b). To test this hypothesis, we computed Kendall's tau (τ) (Kendall, 1955) for our adjusted sequences and the sequence found by Stevenson (1924). Kendall's τ measures the cross-tabulation correspondence between two rankings. Values for τ can vary between 1 (perfect agreement between the rankings) and −1 (perfect disagreement). A value of 0 indicates independence. We chose Kendall's tau-b statistic to enable adjustments for ties within the rankings.

Second, we compared the taxa that were investigated by other authors and by us. There are important methodological problems in comparing growth plate closure sequences of different authors. First, closure information might differ greatly when looking at dry bones, X-rays, or cleared and stained wet specimens (Bull & Payne, 1982). Second, the definition of the moment of closure varies considerably among authors, and it is difficult to find agreements among the authors or the definition is omitted (Bull & Payne, 1982). Moreover, sequences are studied at different taxonomic levels (species, genera, families). Consequently, data obtained from the literature were not included in all our analyses and were used to address in general the issue of variability among clades and to establish whether differences in data acquisition have a significant influence on the computed closure sequence. The taxa chosen for this comparison were *Papio* spp., *Macaca mulatta*, *Pan troglodytes* and *Saimiri sciureus*. Moreover, we compared some sequences from the literature between each other using Kendall's τ. When possible, we calculated the sequences of fusion with the data provided by the literature, but considering our definition for the fusion stage. If this was not possible, the sequences were used as provided. All sequences obtained from other authors were adjusted as already described, and missing growth plates were removed from the analysis.

Statistical analyses were conducted with version 20.0.0; IBM SPSS statistics (Armonk, NY, USA). Clades with reduced bones (e.g. *C. capreolus* and *Equus* spp.) were excluded from Kendall's τ, Kendall's W and ANOVA. Due to the great number of statistical tests in this study, we applied the false discovery rate procedure (FDR) (Benjamini & Hochberg, 1995; Curran-Everett, 2000) to resulting p-values of Kendall's τ, Kendall's W and ANOVA using R, version 2.15.1 (R Foundation for Statistical Computing, Vienna, Austria). This procedure was applied because multiple comparisons can lead to a higher rate of false-positive results, that is, statistically significant differences that are actually not different. The FDR analysis is a method to discover these false positives.

Heterochrony analysis

We performed Parsimov-based genetic inference (PGi) (Harrison & Larsson, 2008) to analyse heterochronic changes in growth plate closure sequences. PGi examines the sequence as one single, complex character and uses the Parsimov algorithm as an edit-cost function to optimize ancestral states and sequence heterochronies. PGi was computed using R, version 2.15.1, and the package pgi 2.0. The analysis included all 15 growth plates and 34 genera. The parameters (Harrison & Larsson, 2008) used for the analysis were as follows: number of

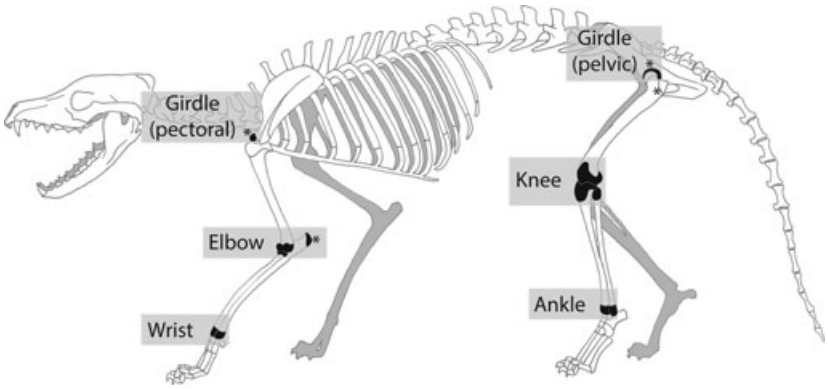

Fig. 2 Schematic drawing of the skeleton of a *Thylacinus cynocephalus*. Epiphyses and growth plates (black) investigated here and their correspondence with anatomical regions are indicated. Pectoral girdle: coracoid process of the scapula; pelvic girdle: acetabulum of the pelvis; elbow: distal humerus, proximal ulna and proximal radius; wrist: distal radius and distal ulna; knee: distal femur, proximal tibia and proximal fibula; ankle: distal tibia and distal fibula. Asterisks indicate 'traction epiphyses' (Parsons, 1904; Serrano *et al.*, 2011): coracoid of the scapula, proximal ulna, acetabulum of the pelvis and greater trochanter of the femur. All other epiphyses are 'pressure epiphyses' (Parsons, 1904; Serrano *et al.*, 2011).

sequences per cycle: 150; number of cycles: 150; and number of sequences retained at each node: 150. Semi-exhaustive search with a limited number of permutations per cycle (max. 10 000) was performed. The PGi analysis was computed eight times independently, and subsequently, the shortest tree, which is assumed to recover the most probable heterochronies, was chosen. Further, event pairing and Parsimov (Jeffery *et al.*, 2005) as described by Koyabu *et al.* (2011) and a continuous analysis (Germain & Laurin, 2009) were conducted to compare the results. Event pairing was computed using R, version 2.15.1, and the continuous analysis was computed using Mesquite, version 2.75 (Maddison & Maddison, 2011), and the PDAP:PDTREE module, version 1.16 (Midford *et al.*, 2011). The phylogeny and branch length estimates were computed as described above.

Growth plate closure and relative age

To track growth plate closure throughout life history, the relative closure state of every specimen with known IDAS was calculated. IDAS was used as a proxy for relative individual age. The relative closure state per specimen was computed as the number of closed growth plates divided by the total number of scored growth plates in that specimen (12, 13 or 15, see above). One-way ANOVA was performed, and IDAS was used as the independent variable.

Results

General patterns of fusion

Examining the raw closure sequences for each main mammalian group, we found that in marsupials, the closure of the sutures of the girdles (i.e. coracoid process of the pectoral girdle and acetabulum of the pelvic girdle) occurs in all species examined before the closure of the growth plates at the elbow (i.e. distal humerus, proximal ulna and radius) (Table S2). *Tachyglossus aculeatus* has the opposite sequence (Table S2). In placentals, the position of the girdles within the closure sequence is variable (Table S2). However, it is always an element of the girdles or the elbow that is ossified first. In placentals, 20.83% of the taxa (distributed randomly) have a girdle element ossifying first; 41.67% (distributed randomly) has an elbow element ossifying first; in the remaining 37.5%, both elements ossify first, which could be by simultaneous closure or unresolved because of the coarseness of data.

In about 56% of investigated taxa (distributed randomly), all elbow elements fuse before any knee element. In the remaining 44% of taxa, there is a partial overlap of fusion events in the elbow and the knee. However, in none of the taxa do any knee elements fuse before an elbow element (Table S2). A similar pattern exists at the ankle and the wrist. In 62.5% of investigated taxa (distributed randomly), excluding *Equus* spp. and *C. capreolus*, all ankle elements fuse before the wrist elements. In the remaining taxa, there is an overlap of fusion events in the ankle and the wrist. In none of the taxa do the wrist elements fuse before the ankle elements (Table S3). Moreover, wrist and ankle elements generally fuse relatively late in the ontogeny. The position of 'tension epiphyses' and 'pressure epiphyses' within the sequences is variable (Tables S2 and S3).

One-way ANOVA of adjusted ranks of all taxa showed that the mean relative closure scores are significantly different among the growth plates examined ($F_{14,479} = 52.629$, $P < 0.0001$) (Fig. 3). This result indicates that approximately the same growth plates are closed early or late in all mammals examined, that is,

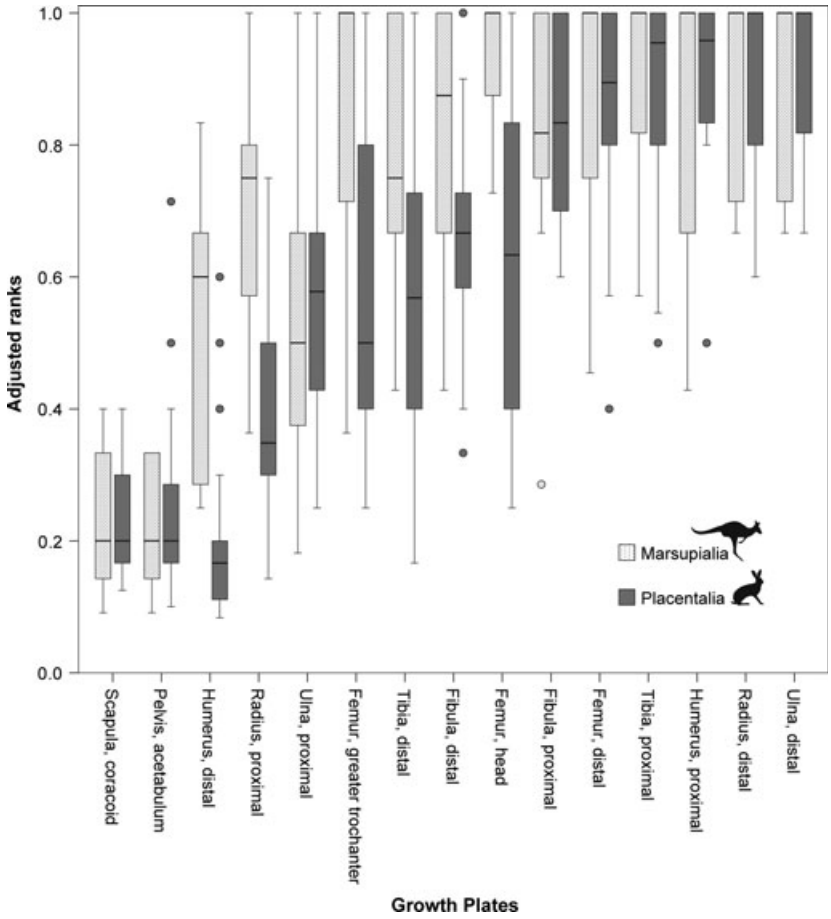

Fig. 3 Adjusted rank ranges (r/r_{max}) of single growth plates across species and genera for marsupials (dotted) and placentals (grey). Ranks range from 0 (fusing first) to 1 (fusing last).

the growth plate closure sequences are rather similar. One-way ANOVA between major clades detected significant differences in the closure scores of the distal humerus, proximal radius, femoral head and femoral greater trochanter in placentals compared with marsupials (Table 3). The same conclusion was obtained after the FDR analysis. Differences in closure scores were also reported in major clades within placentals (Table 3). However, after applying FDR analyses, the only difference that remained statistically significant was the closure score of the greater trochanter of the femur in the comparison of 'Atlantogenata' with Boreoeutheria. In a second step, the confidence intervals of the adjusted closure scores at the nodal values of the last common ancestors of marsupials and placentals were compared to correct the scores for phylogenetic relatedness of the terminal clades (Meslin *et al.*, 2012). The mean relative closure scores remained significantly different among the growth plates examined using one-way ANOVA ($F_{14,59} = 5.419$, $P < 0.0001$). However, two-tailed t-tests (similar variance) detected no significant differences in the closure scores of growth plates between marsupials and placentals.

There are also significant differences in adjusted ranks within clades, often taking together growth plates that are included in one joint and comprise anatomical regions (Washburn, 1946) (Table 4). Girdle elements ossify simultaneously and at a different point in time than all other growth plates in all investigated major mammalian clades (Table 4). Growth plates of the elbow ossify simultaneously in marsupials, but not in placentals (Table 4). In addition, the growth plates of the elbow in marsupials, and the wrist, proximal femur, knee and ankle in marsupials and placentals fuse simultaneously, but they also fuse at a similar time as the growth plates of other anatomical regions. For example, elements of the elbow in marsupials fuse at the same time, but also at the same time as elements of the ankle (Table 4). The sequence of fusion of anatomical regions in marsupials and placentals is different. The simultaneous fusion of growth plates included in an anatomical region was also found in the subgroups (Boreoeutheria, 'Atlantogenata', Laurasiatheria, Euarchontoglires) in approximately the same sequence. Only in 'Atlantogenata' do the ankle elements not fuse simultaneously.

Conservation of growth plate closure sequences

The sequences are different as shown by the analysis of the sequences across clades with Kendall's W (Table 5): the values for W are relatively low and variable, even though many comparisons of sequence commonality within clades are statistically significantly similar. The most significant similarities were found when the sequences of all 15 growth plates were used to calculate the sequence commonality within clades (Table 5). Except for one comparison ('pressure epiphyses' in Euarchontoglires), all comparisons remained statistically significant after applying FDR analyses.

We summarized literature data about epiphyseal closure sequence from 34 studies (Tables S4 and S5). Comparing data from humans (Stevenson, 1924) to our sequence data for all investigated mammals, most sequences across the mammalian clade – and especially primates – are strongly correlated with the sequence found by Stevenson (1924), although no perfect correlation ($\tau = 1$) was detected (Table 6). All initially statistically significant results stayed significant after applying FDR analyses. The results from other comparisons using Kendall's τ are summarized in Table 7. Using one-way ANOVA and Tukey's b *post hoc* tests, we found that the values for τ that resulted from different comparisons of the sequences from the literature (a–e in Table 7) were not significantly different from each other ($F_{4,65} = 1.830$, $P = 0.082$).

Table 3 Differences in adjusted growth plate closure scores between clades. Results from one-way ANOVA are shown. As a comparison, heterochronic changes computed with Parsimov-based genetic inference (PGi) (Harrison & Larsson, 2008) are given. In contrast to ANOVA, PGi gives hypotheses about the most probable evolutionary direction of heterochronic changes.

	$F_{1,30}$	P	p (FDR)	Direction (PGi)
Marsupialia and Placentalia				
HD	28.299	**< 0.001**	**< 0.001**	Early in placentals
RP	21.618	**< 0.001**	**< 0.001**	No shift
FeH	15.785	**< 0.001**	**< 0.001**	No shift
FeG	7.009	**0.012**	0.045	No shift
'Atlantogenata' and Boreoeutheria				
HD	7.875	**0.011**	0.083	No shift
UP	4.736	**0.042**	0.158	No shift
FeH	6.064	**0.023**	0.115	Early in Boreoeutheria
FeG	21.249	**< 0.001**	**< 0.001**	Early in Boreoeutheria
Laurasiatheria and Euarchontoglires				
RP	7.975	**0.014**	0.210	No shift

FDR, false discovery rate; FeG, femur, greater trochanter; FeH, femur, head; HD, humerus, distal; RP, radius, proximal; UP, ulna, proximal; PGi, Parsimov-based genetic inference. Significantly similar results (on the basis of a 0.95 confidence interval) are indicated with bold font.

Heterochrony analysis

The eight PGi consensus trees had similar lengths of 194, 196 (×2), 197 (×2) and 198 (×3) steps, indicating appropriateness of the used parameters. The shortest tree (194 steps) was chosen as best reflecting possible trends in the closure sequence of growth plates throughout mammalian evolution. Heterochronic shifts in major mammalian clades are shown in Fig. 1. They are partially consistent with findings from the analysis of adjusted closure scores with one-way ANOVA. The

Table 4 Growth plates grouped according to their timing of closure through Tukey's b *post-hoc* tests (on the basis of a 0.95 confidence interval) revealed that the mean adjusted ranks often group growth plates (indicated in grey) that are included in one joint and thus comprise anatomical regions (Fig. 2).

Marsupialia, $n = 9$	1	2	3	4	Placentalia, $n = 22$	1	2	3	4
PA	0.022				HD	0.064			
SC	0.028				SC	0.104			
HD		0.405			PA	0.120			
UP		0.448	0.448		RP		0.280		
RP		0.660	0.660	0.660	UP			0.493	
TD			0.733	0.733	FeG			0.509	
FD			0.766	0.766	FeH			0.565	
FP			0.748	0.748	TD			0.524	
TP				0.844	FD			0.618	
FeD				0.818	FP				0.825
HP				0.815	FeD				0.830
FeG				0.799	TP				0.859
FeH				0.932	HP				0.887
RD				0.863	RD				0.885
UD				0.852	UD				0.897

n, number of genera; FD, fibula, distal; FP, fibula, proximal; FeD, femur, distal; FeG, femur, greater trochanter; FeH, femur, head; HD, humerus, distal; HP, humerus, proximal; PA, pelvis, acetabulum; RD, radius, distal; RP, radius, proximal; SC, scapula, coracoid; TD, tibia, distal; TP, tibia, proximal; UD, ulna, distal; UP, ulna, proximal.

Table 5 Concordance between growth plate closure sequences within clades and locomotor groups using Kendall's W.

		All growth plates (n = 15)		Anatomical regions (n = 5)		'Pressure epiphyses' (n = 11)		'Traction epiphyses' (n = 4)	
	N	Kendall's W	P (p(FDR))	Kendall's W	P (p(FDR))	Kendall's W	P (p(FDR))	Kendall's W	P (p(FDR))
Clades									
Mammalia	32	0.268	**< 0.001 (< 0.001)**	0.145	0.869 (1.000)	0.295	**< 0.001 (< 0.001)**	0.165	0.926 (1.000)
Placentalia	22	0.248	**< 0.001 (< 0.001)**	0.191	0.519 (1.000)	0.248	**< 0.001 (< 0.001)**	0.127	0.969 (1.000)
Marsupialia	9	0.436	**< 0.001 (< 0.001)**	0.305	0.142 (0.887)	0.456	**< 0.001 (< 0.001)**	0.394	0.126 (0.608)
'Atlantogenata'	6	0.275	**0.001 (0.002)**	0.248	0.286 (1.000)	0.143	0.165 (0.186)	0.441	0.117 (0.608)
Boreoeutheria	16	0.196	**< 0.001 (< 0.001)**	0.148	0.744 (1.000)	0.216	**0.002 (0.007)**	0.07	0.997 (1.000)
Laurasiatheria	4	0.081	0.301 (0.319)	0.105	0.665 (1.000)	0.23	0.055 (0.010)	0.056	0.879 (1.000)
Euarchontoglires	12	0.211	**< 0.001 (< 0.001)**	0.106	0.885 (1.000)	0.175	**0.031** (0.080)	0.077	0.985 (1.000)
Carnivora	3	0.043	0.526 (0.526)	0.013	0.939 (1.000)	0.261	0.057 (0.010)	0.083	0.717 (1.000)
Rodentia	5	0.263	**0.003 (0.006)**	0.000	1.000 (1.000)	0.161	0.132 (0.158)	0.165	0.62 (1.000)
Primates	6	0.200	**0.010 (0.015)**	0.131	0.656 (1.000)	0.176	0.085 (0.118)	0.051	0.962 (1.000)
Dasyurumorphia	3	0.186	0.061 (0.084)	0.015	0.926 (1.000)	0.115	0.281 (0.281)	0.500	0.135 (0.608)
Diprodontia	3	0.318	**0.008 (0.014)**	0.380	0.15 (0.887)	0.519	**0.003 (0.009)**	1	**< 0.001 (< 0.001)**
Locomotor groups									
Semi-aquatic	3	0.308	**0.01 (0.015)**	0.000	1.000 (1.000)	0.121	0.264 (0.280)	0.175	0.497 (1.000)
Terrestrial	10	0.365	**< 0.001 (< 0.001)**	0.146	0.682 (1.000)	0.322	**< 0.001 (< 0.001)**	0.183	0.678 (1.000)
Cursorial	3	0.502	**0.001 (0.002)**	0.013	0.939 (1.000)	0.246	0.067 (0.101)	< 0.001	1.000 (1.000)
Arboreal	3	0.130	0.143 (0.171)	0.108	0.584 (1.000)	0.255	0.061 (0.010)	0.083	0.717 (1.000)
Semi-fossorial	3	0.180	0.067 (0.086)	0.450	0.105 (0.887)	0.209	0.100 (0.129)	< 0.001	1.000 (1.000)
Scansorial	6	0.081	0.301 (0.319)	0.293	0.197 (0.887)	0.196	0.056 (0.010)	0.114	0.810 (1.000)

FDR, false discovery rate; N, number of compared clades/group; n, number of compared growth plates.
Significantly similar results (on the basis of a 0.95 confidence interval) are indicated with bold font.

complete set of shifts is shown in Fig. S1. Parsimov did not detect any shifts in deep nodes that represent major clades (results not shown).

The continuous analysis detected only few marsupial and placental taxa that fall outside the 95% confidence interval of Theria. No clade, including major clades within Placentalia, is represented by an outstanding number of species that deviate from the 95% confidence interval of Theria. The evolutionary rates of the growth plates of Marsupialia and Placentalia are shown in Table 8.

Growth plate closure and relative age

When comparing the relative number of closed growth plates in marsupials and placentals, the differences in the mean values are significantly different among the IDAS in placentals ($F_{3,136} = 6.25$, $P = 0.001$), but not in marsupials ($F_{3,48} = 2.591$, $P = 0.064$) (Fig. 4). In 'Atlantogenata' ($F_{2,16} = 0.682$, $P = 0.522$) and Laurasiatheria ($F_{1,14} = 2.958$, $P = 1.109$), there is no trend, whereas in Euarchontoglires ($F_{3,104} = 7.207$, $P < 0.0001$) and Boreoeutheria ($F_{3,119} = 7.820$, $P < 0.0001$), there is a significant positive trend. The positive trend in placentals might even be underestimated because we did not score specimens with all growth plates closed. This would have resulted in an increased number of high values for relative number of closed growth plates in late adult or senile specimens and probably a greater positive trend. The positive trend in placentals indicates that when increasing the relative age, more growth plates are closed. Unexpectedly, there is no such trend in marsupials. Moreover, with the exception of one single specimen of *T. cynocephalus* (NHM 1963.8.30.1), no marsupial specimen has all the growth plates closed in our sample, even in late adult or senile stages. In contrast, we observed while sampling that closure of all growth plates (100% relative closure) can be observed in numerous placental specimens (e.g. various specimens of *Dendrohyrax* spp., *Myrmecophaga* spp., *O. afer*, *Equus* spp., *C. capreolus*, *Felis silvestris*, *Castor fiber*, *D. leporina*, *M. marmota*, *P. troglodytes*, *Papio* spp.). However, the marsupial pattern is not homogeneous: different growth plates were found with obliterated or lapsed (never completed) union (Table S6). Growth plates that close completely in all investigated species are the coracoid of the scapula, the acetabulum of the pelvis, the proximal radius and the distal humerus (Table S6).

Discussion

In this study, we investigated the sequence of epiphyseal growth plate union in the limb bones of diverse mammalian taxa. The influence of phylogenetic relationships and biomechanical factors on the commonality of sequences was analysed, and the relation of growth plate closure to relative age was established. Although the

Table 6 Concordance in growth plate closure sequences of taxa investigated in this study compared with the human sequence by Stevenson (1924) using Kendall's τ.

Genera	Kendall's τ	*P*	p (FDR)
Papio spp.	0.943	**< 0.001**	**< 0.001**
Saimiri spp.	0.876	**< 0.001**	**< 0.001**
Pan troglodytes	0.856	**< 0.001**	**< 0.001**
Perodicticus potto	0.845	**< 0.001**	**< 0.001**
Macaca mulatta	0.841	**< 0.001**	**< 0.001**
Felis silvestris	0.838	**< 0.001**	**< 0.001**
Chlorocebus aethiops	0.826	**< 0.001**	**< 0.001**
Heterohyrax brucei	0.807	**< 0.001**	**< 0.001**
Erinaceus europaeus	0.780	**< 0.001**	**< 0.001**
Dasyurus spp.	0.757	**< 0.001**	**< 0.001**
Lepus europaeus	0.742	**< 0.001**	**< 0.001**
Procavia capensis	0.735	**0.001**	**0.002**
Hydrochoeris hydrochaeris	0.723	**0.001**	**0.002**
Dorcopsis muelleri	0.716	**0.001**	**0.002**
Castor fiber	0.713	**0.001**	**0.002**
Capreolus capreolus	0.701	**0.002**	**0.003**
Tenrec ecaudatus	0.688	**0.002**	**0.003**
Dasyprocta leporina	0.686	**0.001**	**0.002**
Ursus arctos	0.681	**0.001**	**0.002**
Marmota marmota	0.674	**0.002**	**0.003**
Dasypus spp.	0.658	**0.003**	**0.004**
Macropus spp.	0.636	**0.002**	**0.003**
Myocastor coypus	0.622	**0.003**	**0.004**
Didelphis spp.	0.613	**0.005**	**0.006**
Orycteropus afer	0.592	**0.005**	**0.006**
Isoodon macrourus	0.578	**0.013**	**0.017**
Myrmecophaga tridactyla	0.483	**0.026**	**0.032**
Perameles spp.	0.478	**0.033**	**0.036**
Thylacinus cynocephalus	0.467	**0.028**	**0.033**
Phoca vitulina	0.442	**0.031**	**0.035**
Sarcophilus harrisii	0.368	0.079	0.084
Tachyglossus aculeatus	0.283	0.178	0.184
Phascolarctos cinereus	0.056	0.806	0.806

FDR, false discovery rate.
Significant results are indicated with bold font.

Table 7 Comparisons (a–e) of own sequence data and sequence data from the literature using Kendall's τ.

	Comparisons	Range of Kendall's τ (mean)
(a)	Taxa from the present study in comparison with Stevenson's (1924) data for humans (Table 6)	0.056–0.943 (0.658)
(b)	Same taxon from the present study and the literature	0.568–0.933 (0.769)
(c)	Different sexes from the same taxon (literature)	0.490–0.982 (0.732)
(d)	Taxa from the literature in comparison with Stevenson's (1924) data for humans: authors who claim that the closure sequence is a mammalian universal	0.742–0.876 (0.812)
(e)	Taxa from the literature in comparison with Stevenson's (1924) data for humans: authors who claim that the closure sequence is variable	0.245–0.846 (0.612)

Table 8 Evolutionary rates (changes per million years) of the timing of closure of different growth plates in the limb bones of marsupials and placentals.

Growth plates	Marsupialia	Placentalia
PA	8.17×10^{-05}	3.53×10^{-04}
SST	2.95×10^{-04}	2.04×10^{-04}
FeH	4.97×10^{-04}	1.27×10^{-03}
RD	8.09×10^{-04}	4.34×10^{-04}
DU	8.16×10^{-04}	3.22×10^{-04}
HD	8.75×10^{-04}	2.09×10^{-04}
TP	1.17×10^{-03}	4.72×10^{-04}
TD	1.19×10^{-03}	1.27×10^{-03}
UP	1.25×10^{-03}	4.44×10^{-04}
RP	1.45×10^{-03}	6.21×10^{-04}
FP	1.56×10^{-03}	5.83×10^{-04}
FD	1.86×10^{-03}	7.06×10^{-04}
FeD	2.45×10^{-03}	7.81×10^{-04}
HP	2.78×10^{-03}	3.54×10^{-04}
FeG	2.84×10^{-03}	9.24×10^{-04}

FD, fibula, distal; FP, fibula, proximal; FeD, femur, distal; FeG, femur, greater trochanter; FeH, femur, head; HD, humerus, distal; HP, humerus, proximal; PA, pelvis, acetabulum; RD, radius, distal; RP, radius, proximal; SC, scapula, coracoid; TD, tibia, distal; TP, tibia, proximal; UD, ulna, distal; UP, ulna, proximal.

sequence of growth plate closure is not conserved across mammals, we identified conserved closure patterns and similar timing of closure in anatomical regions. First, few heterochronic shifts (change of timing) characterize major mammalian clades. Second, biomechanical factors (locomotor group affiliation) have no coherent influence on the sequence of closure. Third, most examined growth plates of marsupials have a higher evolutionary rate than the same growth plates in placentals. Fourth, marsupials keep some of their epiphyses separated during their entire life, in contrast to placentals.

Growth plate closure sequences in a phylogenetic context

Stevenson (1924), as reported also by many other authors (Dawson, 1925; Todd, 1930a,b; Koch, 1935; Todd & Todd, 1938), claimed that the sequence of epiphyseal union was a general and uniform mammalian trait. We tested this statement by examining raw closure sequences and using Kendall's τ and Kendall's W. Some patterns of closure were similar in all investigated species and genera (Fig. 3, Tables S2 and S3). The sequence commonality (using Kendall's W) ranged from almost no correlation to very strong correlation, whereas the mean correlation was medium to strong (Table 5). Similar values of correlation (medium to very

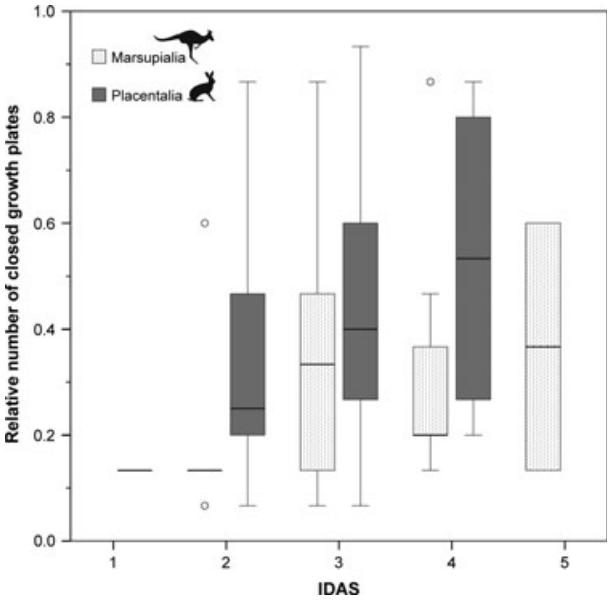

Fig. 4 Relative number of closed growth plates in marsupials (dotted) and placentals (grey) of different individual age, indicated as individual dental age stages (IDAS) (generalized individual dental age stages) (Anders *et al.*, 2011). IDAS 2, juvenile; IDAS 3, adult; IDAS 4, late adult; IDAS 5, senile. Notice that we reported no marsupials with IDAS 1 and no placentals with IDAS 5.

strong) resulted from comparing between the literature data and our data against the sequence found by Stevenson (1924) using Kendall's τ (Table 6, a in Table 7). However, the values for τ in the sequences found by the authors who claim that the growth plate closure sequence is a mammalian universal (d in Table 7) are not more similar to Stevenson's (1924) sequence than the sequences found by authors who propose that there is variability across Mammalia (e in Table 7). Moreover, the differences between sexes can be greater than the differences between taxa (c in Table 7). The comparisons between same taxa from the present study (b in Table 7) and the literature resulted in similar values for τ when comparing different sexes or taxa to one another, suggesting that possible errors due to different observers and definitions of growth plate closure do not result in greater sequence differences than can be observed between different sexes or taxa. Therefore, the comparisons between the different literature sources are feasible.

Concluding, our sequences and those from the literature suggest that growth plate closure sequence is not a mammalian universal, even though some consistencies exist. In particular, the strong correlation of Kendall's τ between humans (Stevenson, 1924) and the primates investigated in this study (Table 6), as well as the strong correlation of Kendall's W within major mammalian clades (Table 5), suggests phylogenetic influence on growth plate union. Fossil evidence further confirms that growth plate closure sequences did evolve considerably through time. The growth plates that connect the coracoid to the scapula as well as ilium, ischium, and pubis together are unfused in the Jurassic dryolestoid *Henkelotherium guimarotae*, whereas almost all growth plates of the long bones, with the exception of the distal tibia and fibula, are completely fused (Krebs, 1991). This is a remarkable deviation from the general early fusion of growth plates of the girdles in the extant mammals investigated here.

The heterochrony method PGi has revealed many shifts in young taxa, whereas there are only few changes in deeper nodes (Fig. 1, Fig. S1). Additionally, the continuous analysis detected only few marsupials and placentals outside the 95% confidence interval of Theria. These taxa represent different clades and therefore indicate that heterochronic shifts do not characterize major clades, but rather young taxa. Germain & Laurin (2009) explained similar findings from other authors as a methodological artefact; they detected that type I error (false-positive) rates on terminal branches are higher than on internal branches when using heterochrony analyses such as Parsimov or continuous analysis. Therefore, it is pointless to speculate about possible adaptive significance of all these changes in all investigated taxa, and we will restrict this discussion to the heterochronic shifts in major mammalian clades. However, most differences in adjusted closure scores between major clades disappeared when we corrected the adjusted closure scores for the phylogenetic relatedness of the terminal taxa, that is, when comparing the confidence intervals of the last common ancestor of marsupials and the last common ancestor of placentals. The observed changes between clades in the data that were not corrected for phylogenetic relationship do therefore probably not characterize deep nodes but rather 'families', genera or species. This would imply that the observed pattern is not a methodological artefact, but rather a biological phenomenon. This finding is consistent with the hypothesis that modifications during early development, such as cartilage formation, lead to major phenotype alterations, for example, digit reduction, whereas modifications in the later development and growth, such as ossification, lead to differences in skeletal features between species of the same genus or closely related genera (Farnum, 2007).

Differing from the universal altriciality of marsupials, placentals show an extensive range of neonatal maturity and gestation length that expose the growing organism to differing selection pressures. These variations in life-history traits often coincide with heterochrony. In marsupials, the forelimbs are more developed than the hindlimbs by the time of birth, resulting from an acceleration in early development of the former and a delay in early development and ossification of the latter (Weisbecker *et al.*, 2008; Sears, 2009; Sears *et al.*, 2012). This phenomenon is

crucial for the young being able to crawl to the pouch of the mother and is probably caused by 'trade-offs' in energy allocation (Weisbecker *et al.*, 2008). Moreover, it has traditionally been presumed and empirically tested (Sears, 2004; Bennett & Goswami, 2013) that the developmental constraints due to the specialized and conserved marsupial mode of reproduction lead to relatively low anatomical diversity in comparison with placentals. In contrast, Sánchez-Villagra (2013) argued that these developmental biases can be – and have often been observed to actually be – circumvented. The observed smaller morphospace and lower taxonomical diversity in marsupials might be more a reflection of a lack of ecological opportunity and a multitude of factors involving geographical and physiological variables (Sánchez-Villagra, 2013). In fact, we are not able to recognize developmental constraints with our data. Instead, we found that in marsupials, 12 of the 15 growth plates investigated have higher evolutionary rates of heterochronic shifts than in placentals, whereas only three growth plates have a higher rate in placentals (Table 8).

We expected that the peculiar mode of prenatal development of marsupials might have also consequences for the post-natal growth: in marsupials, growth plates in the hindlimbs might stay open for a longer period of time than in the forelimbs to allow subsequent growth. However, the only heterochronic shift that concerns the hindlimbs in marsupials is a delay in the closure of the distal fibula (Fig. 1). This delay could be the result of the hindlimbs growing for a longer period of time than the forelimbs to catch up with the already highly developed forelimbs. However, the distal growth plate of the tibia does not shift in a similar direction. Significant results from ANOVA (not detected using PGi) indicated possible heterochronic shifts in growth plates of the proximal femur (Table 3). However, the direction of the shift and the clade in which the shifts most probably occurred are not known. We conclude that the heterochronic shifts in growth plate closure is not a function of the onset of ossification. There is no causal relationship between the two. Moreover, the detected higher evolutionary rate in marsupials, together with the hypothesis that shifts in the growth plate closure sequence are characteristic for young taxa rather than for major clades, indicates that discrepancies between the life history of marsupials and placentals are not mirrored by the sequence of epiphyseal growth plate closure.

The placental groups Afrotheria and Xenarthra (together 'Atlantogenata') are characterized by ontogenetic peculiarities not found in boreoeutherians, for example, late dental eruption, unusually rapid growth to adult size and unusually variable vertebral formulae (Asher *et al.*, 2009). Therefore, we wondered whether members of this clade might also share a peculiar pattern of growth plate closure. Even though we detected several changes using ANOVA, PGi showed that these changes resulted from changes within Boreoeutheria (Table 3). Shifts in the fore- and hindlimbs are on the branch leading to Boreoeutheria and within Boreoeutheria to Euarchontoglires (Fig. 1). No shifts characterize 'Atlantogenata' and Laurasiatheria (Fig. 1). As the terminal taxa within Boreoeutheria and 'Atlantogenata' are highly diverse, we can think of no adaptive hypothesis explaining these shifts.

Growth plate closure sequences in a biomechanical context

The functional aspects that may lead to heterochronic shifts in the growth plate closure sequence include the function of single joints adjacent to the growth plates. This aspect is important regarding mechanical loadings. Stresses are assumed to strongly influence musculoskeletal growth and cause the skeleton to alter its shape to cope with physical demands (Carter *et al.*, 1991). The different epiphyses and growth plates of bones are discriminated in 'traction epiphyses' and 'pressure epiphyses' (Parsons, 1904; Serrano *et al.*, 2011) (Fig. 2). 'Pressure epiphyses' are positioned perpendicular to the bone axis and are compressed by the gravitational force and reaction force of the adjacent diaphysis (Serrano *et al.*, 2011). Due to this compression and the movement of the joints, 'pressure epiphyses' are under hydrostatic and shear stresses (Serrano *et al.*, 2011). Arkin & Katz (1956), Carter & Wong (1988), Carter *et al.* (1991, 1998) found that these stresses decrease the rate of epiphyseal cartilage growth. 'Traction epiphyses' provide attachment for tendons and muscles and are subject to lateral stresses and tension (Parsons, 1904; Serrano *et al.*, 2011). These lateral forces cause high shear stresses, which are assumed to promote ossification (Arkin & Katz, 1956; Porter, 1978; Breburda *et al.*, 2001).

Because of these different influences of stress components on cartilage maintenance, growth and ossification, it seems likely that the growth plate closure sequences of members of one locomotor group are more similar to one another than they are to sequences of taxa from other locomotor groups. Moreover, the sequences of closure might be more similar when examining these two growth plate types separately (Serrano *et al.*, 2011).

We found no significant greater concordance in growth plate closure sequences within locomotor groups (Tables 2 and 5) than within clades (Table 5). Investigating the special case of bipedal locomotion in which the forelimb and all its epiphyses are free from gravitational pressure, we failed to find high accordance in the sequences of the bipedal genera (*Dorcopsis* spp. and *Macropus* spp.) and the sequence found in humans (Stevenson, 1924) (Table 6). Lack of accordance might be coupled with the 'five-footed' gait in *Macropus* spp.

during grazing or browsing: they balance on tail and forelegs while swinging their hindlimbs forward (Nowak, 1999). This peculiar mode of locomotion loads forelimbs and tail with gravitational pressure. Growth plates of human arms, on the other hand, are constantly under tension due to their own weight and carrying items. However, we did not find heterochronic changes in growth plate fusion associated with biomechanical stresses due to bipedality.

Comparing the sequences of 'tension epiphyses' and 'traction epiphyses' separately does not result in more concordance among clades and locomotor groups than when comparing all growth plates together (Table 5). Our result contradicts the findings by Serrano *et al.* (2011) who reported perfect concordance in growth plate closure sequence in different artiodactyls when distinguishing between these two types of epiphyses.

Anatomical regions

Using ANOVA and post- hoc tests, we found that the growth plates of anatomical regions (girdles, elbow, wrist, proximal femur, knee, ankle) (Fig. 2) are more integrated than two growth plates of the same bone (Table 4). We confirm previous findings about single clades (Washburn, 1946; Hofer *et al.*, 1956; Tappen & Severson, 1971; Cheverud, 1981). However, as stated for New World monkeys by Tappen & Severson (1971), there appears to be overlap of the sequence of union between regions.

Complete growth plate closure and lapsed union

Whereas complete closure of all growth plates in late adult and senile specimens could be observed in many placental species, this is not the case for marsupials. Additionally, increasing age involves an increased number of closed growth plates in placentals, whereas no correlation was found in marsupials (Fig. 4). Lacking of growth plate union in marsupials has previously been reported by Washburn (1946) and Lowrance (1949). Incomplete fusion of growth plates in placentals is known for some species, including rat (Dawson, 1925, 1934; Mullender *et al.*, 1996; Roach *et al.*, 2003) and mouse (Dawson, 1935). There is further some evidence for incomplete fusion of growth plates in the xenarthran *Dasypus hybridus* (Ciancio *et al.*, 2012) and in other members of 'Atlantogenata' (S. Werning, personal communication). These findings suggest that marsupials might continue growing throughout life, a hypothesis that is supported by Lowrance (1949). This author found that the variability in the bone lengths of the opossum exceeds that of placentals and suggested that certain parts of the opossum skeleton continue growing throughout life. Furthermore, histological data suggest that some marsupials do not generally reach skeletal maturity before death (S. Werning, personal communication). Contrastingly, longitudinal bone growth stops in rats, even though the cartilaginous growth plate endures until late adulthood (Roach *et al.*, 2003). These findings raise the question whether the condition found in marsupials and some placentals is primitive or derived.

The secondary ossification centres are usually tied to the evolution of determinate growth. Calcified and bony epiphyses evolved several times independently in many vertebrate lineages: Teleostei, Anura, Lepidosauria, Theria and possibly Aves (Haines, 1942; Carter *et al.*, 1998). Although bony epiphyses have been reported in some kannemeyeriid dicynodonts, a group of nonmammaliaform synapsids (Walter, 1985), the lack of secondary ossification centres was hypothesized to be the plesiomorphic condition in cynodonts and mammaliaforms (Luo *et al.*, 2007). Martin (2005) studied several long bones with different sizes of the basal mammaliaform *Haldanodon exspectatus* (Docodonta). This author reported the absence of secondary ossification centres and suggested that this finding might indicate indeterminate growth. Martin (2005) also reported open sutures between the scapula and the coracoid in *Haldanodon*. Although we analysed the growth plates in the long bones together with the sutures within the girdles (i.e. between scapula and coracoid as well as between pubis, ilium, and ischium), they have a different origin. The girdle sutures connect primary ossification centres, whereas the growth plates in the long bones connect primary and secondary ossification centres. Thus, the question about the evolution of the secondary ossification centres remains unresolved when the sutures of the girdles are analysed.

Secondary ossification centres and growth plates have been found in the long bones of several Mesozoic mammals: several multituberculate species (Kielan-Jaworowska & Gambaryan, 1994; Hurum & Kielan-Jaworowska, 2008), the spalacotherioid *Zhangheotherium quinquecuspidens* (Luo & Ji, 2005), the dryolestoid *H. guimarotae* (Krebs, 1991) and *Vincelestes neuquenianus*, a close relative of Theria (Rougier, 1993). Moreover, secondary ossification centres are known from extant monotremes. All specimens show different levels of fusion, including the complete fusion stage. These data suggest that secondary ossification centres may have been present in the last common ancestor of crown mammals.

Alternatively, the absence of secondary ossification centres in *Jeholodens jenkinsi* (Triconodonta) (Ji *et al.*, 1999), *Yanoconodon allini* (Triconodonta) (Luo *et al.*, 2007), *Gobiconodon ostromi* (Triconodonta) (Jenkins & Schaff, 1988), *Volaticotherium antiquum* (Meng *et al.*, 2006), and *Akidolestes cifellii* (Spalacotherioidea) (Li & Luo, 2006) might indicate either skeletal maturity of the specimens and determinate growth, or the complete lack of secondary ossification centres, as suggested by Luo *et al.* (2007). The second possibility implies that

secondary ossification centres have evolved twice in crown mammals: in Monotremata and Theria.

Regarding Montremata, we reported two *T. aculeatus* specimens (NRM A875308 and ZMM MAM 35998), in which all growth plates were closed. Taking this finding into account, there are four possibilities to explain the evolution of secondary ossification centres and determinate growth in extant mammals (Fig. 5a–d). We first report the hypothesis that secondary ossification centres evolved only once in crown mammals (Fig. 5a,b). If the last common ancestor of all living mammals showed lapsed union, complete union might have evolved twice within crown mammals: first, on the lineage leading to modern monotremes, and second, on the lineage leading to Placentalia (Fig. 5a). If so, lapsed union must have reversed among placentals (Fig. 5a). Alternatively, the finding of complete union in monotremes and some extinct mammals suggests that this could represent the primitive mammalian condition, and lapsed union might be a newly evolved trait in marsupials and some placentals (Fig. 5b). The second scenario (Fig. 5b) requires only three steps to explain the observed patterns and is more parsimonious than the first scenario, which requires four steps (Fig. 5a). If secondary ossification centres evolved separately in the lineage leading to extant monotremes and in therians, there is no most parsimonious solution for this problem, either complete or lapsed union could be the ancestral state in Placentalia (Fig. 5c,d). The evidence about the ancestral condition in mammals, that is, complete or incomplete growth plate closure, is not conclusive.

Conclusion

This study shows that there are common patterns of growth plate closure across the mammalian clades, but that there is no single growth plate closure sequence that is uniform in all mammals. Differences in mechanical stresses that act on growth plates have no or only minor influences on the sequences. These findings indicate that growth plate closure sequence is determined more strongly by intrinsic (phylogenetic) factors than by extrinsic (biomechanical) factors. We detected a large number of heterochronic shifts, but they seem to occur in patterns that are not explained by simple functional correlates. We also found that heterochronic shifts in the growth plates of marsupials occur with a higher rate than in placentals. This presents a contrast with the more limited variation in timing and morphospace occupation typical for marsupial development (Sears, 2004; Bennett & Goswami, 2013). Moreover, we found no correspondence between patterns of prenatal and post-natal limb development and growth: one could interpret the heterochronic shifts in post-natal growth in marsupials, in contrast to placentals, as a reaction of post-natal growth otherwise present in prenatal phases of development, as measured in different aspects of organogenesis, chondrogenesis and osteogenesis (Weisbecker *et al.*, 2008; Sears, 2009; Sears *et al.*, 2012). However, we would like to point out that this pattern of developmental morphospace occupation does not necessary reflect a mechanistic or causal mechanism. Instead, it may just be a result of evolutionary

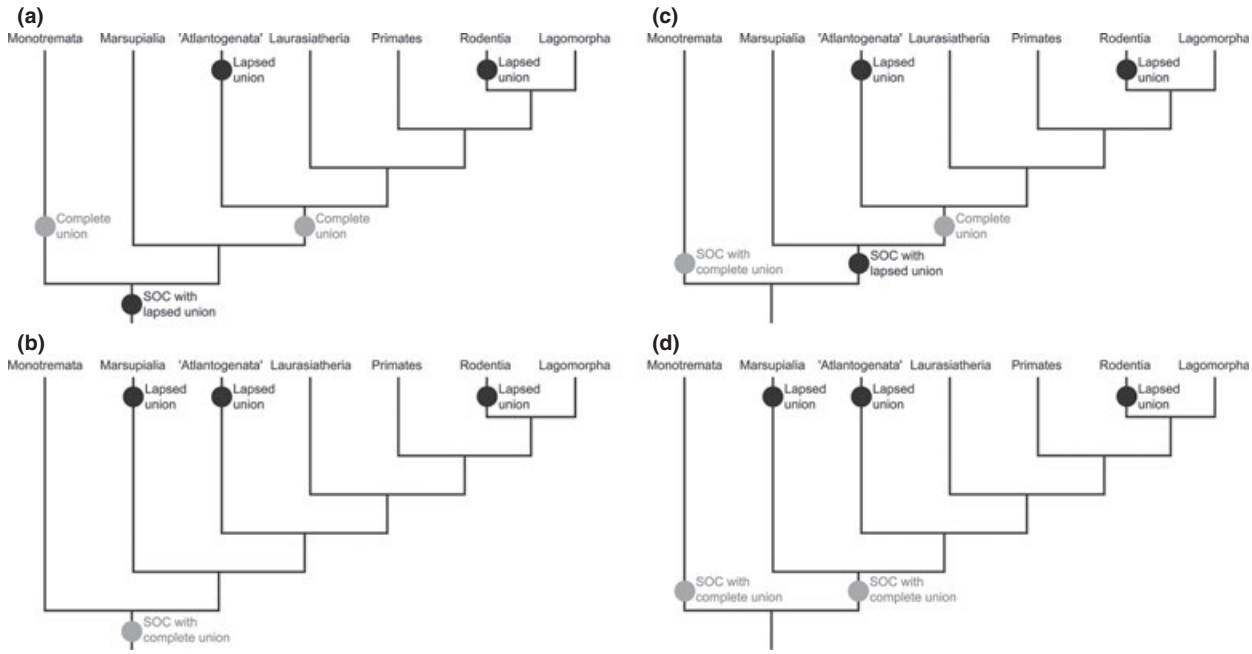

Fig. 5 Four scenarios (a–d) can explain observations of the evolution of secondary ossification centres as well as complete and lapsed growth plate union in mammals.

pathways determined by ecological opportunity (Sánchez-Villagra, 2013).

Even marsupials that are senile have some open growth plates, and there is evidence that marsupials continue growing throughout life. Apart from that, a likely reason for our findings is a decoupling of longitudinal growth from growth plate fusion, as hypothesized for humans by Stevenson (1924), which is probably a widespread if not universal phenomenon in mammals. Recent evidence indicates that growth plate fusion does not cause cessation of growth, but fusion follows cessation of growth (Nilsson & Baron, 2004). Moreover, fusion is not simply the result of continued cartilage replacement, but a different process (Parfitt, 2002). Empirical evidence for a decoupling of cessation of growth and growth plate closure was moreover found by Roach *et al.* (2003). Therefore, growth plate fusion does not necessarily reflect skeletal maturity, and any adaptational conclusions about growth heterochrony that might arise from discovery of patterns of fusion are insubstantial.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Complete set of heterochronic changes in growth plate fusion that was computed using Parsimov-based Genetic Inference (PGi) (Harrison & Larsson, 2008).

Table S1 Species per genus (Wilson & Reeder, 2005) examined in this study.

Table S2 Sequences of growth plate closure

Table S3 Sequences of growth plate closure.

Table S4 Summary of literature data about growth plate closure sequences of different taxa.

Table S5 Growth plate closure sequences of literature data.

Table S6 Marsupial species and genera investigated in this study and growth plates that show lapsed (never completed) union (lu).

Data deposited at Dryad: doi:10.5061/dryad.7b11m

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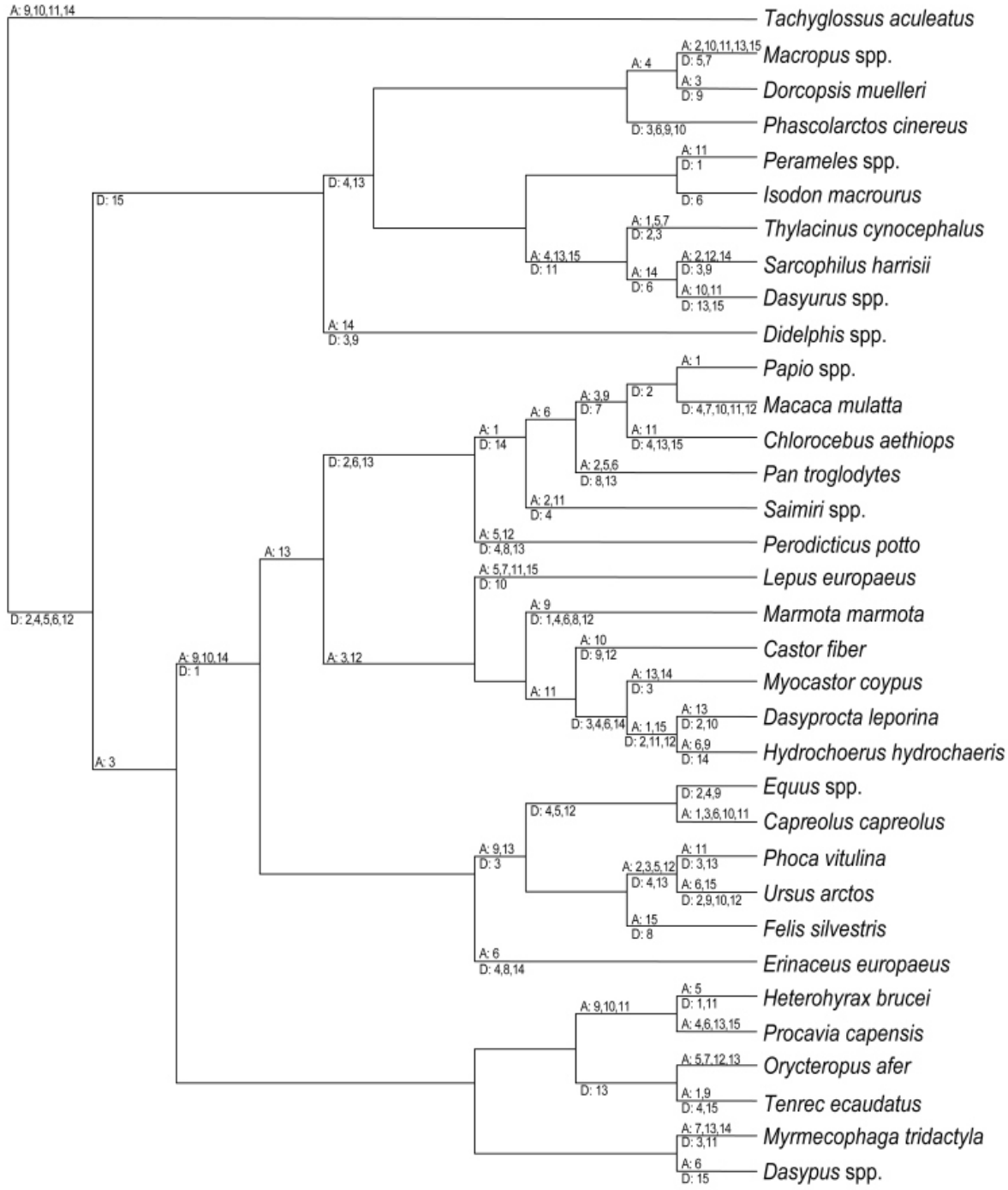

Supplementary Figure S1. Complete set of heterochronic changes in growth plate fusion that was computed using Parsimov-based Genetic Inference (PGi) (Harrison & Larsson, 2008). A: acceleration; D: delay; 1: scapula, coracoid; 2: humerus, proximal; 3: humerus, distal; 4: ulna, proximal; 5: ulna, distal; 6: radius, proximal; 7: radius, distal; 8: pelvis, acetabulum; 9: femur, head; 10: femur, greater trochanter; 11: femur, distal; 12: tibia, proximal; 13: tibia, distal; 14: fibula, proximal; 15: fibula, distal.

Supplementary Table S1. Species per genus (Wilson & Reeder, 2005) examined in this study.

Genus	Species	Genus	Species
Tachyglossus	*T. aculeatus*	*Orycteropus*	*O. afer*
Didelphis	*D. albiventris*	*Tenrec*	*T. ecaudatus*
	D. aurita	*Erinaceus*	*E. europaeus*
	D. marsupialis	*Capreolus*	*C. capreolus*
	D. virginiana	*Equus*	*E. caballus przewalskii*
Dorcopsis	*D. muelleri*		*E. quagga*
Macropus	*M. agilis*		*E. zebra*
	M. eugenii		*E. asinus*
	M. giganteus		*E. burchellii*
	M. parryi		*E. hemionus*
	M. robustus	*Perodicticus*	*P. potto*
	M. rufogriseus	*Chlorocebus*	*C. aethiops*
	M. rufus	*Macaca*	*M. mulatta*
Phascolarctos	*P. cinereus*	*Papio*	*P. anubis*
Thylacinus	*T. cynocephalus*		*P. cynocephalus*
Sarcophilus	*S. harrisii*		*P. hamadryas*
Dasyurus	*D. geoffroii*		*P. papio*
	D. maculatus	*Pan*	*P. troglodytes*
	D. viverrinus	*Saimiri*	*S. oerstedii*
Perameles	*P. bougainville*		*S. sciureus*
	P. gunnii	*Marmota*	*M. marmota*
	P. nasuta	*Castor*	*C. fiber*
Isoodon	*I. macrourus*	*Myocastor*	*M. coypus*
	I. obesulus	*Hydrochoeris*	*H. hydrochaeris*
Dasypus	*D. hybridus*	*Dasyprocta*	*D. leporina*
	D. novemcinctus	*Lepus*	*L. europaeus*
Myrmecophaga	*M. tridactyla*	*Phoca*	*P. vitulina*
Heterohyrax	*H. brucei*	*Ursus*	*U. arctos*
Procavia	*P. capensis*	*Felis*	*F. silvestris*

Supplementary Table S2. Sequences of growth plate closure. Low numbers denote early cssification. Letters are abbreviations for growth plates. FD: fibula, distal; FP: fibula, proximal; FeD: femur, distal; FeG: femur, greater trochanter; FeH: femur, head; HD: humerus, distal; HP: humerus, proximal; PA: pelvis, acetabulum; RD: radius, distal; RP: radius, proximal; SC: scapula, coracoid; TD: tibia, distal; TP: tibia, proximal; UD: ulna, distal; UP: ulna, proximal. Shadings denote anatomical regions: dark grey, girdle; intermediate, elbow; light, knee.

Tachyglossus aculeatus	UP 1	RP 1	SC 2	PA 2	FeD 2	FP 2	HD 3	HP 4	TP 4	FeH 5	FeG 5	UD 6	TD 6	FD 6	RD 7
Didelphis spp.	SC 1	PA 1	UP 2	HD 3	RP 4	TD 4	FP 4	HP 5	UD 5	RD 5	FeH 5	FeG 5	FeD 5	TP 5	FD 5
Dorcopsis muelleri	SC 1	PA 1	HD 2	UP 2	RP 3	HP 4	UD 4	RD 4	FeH 4	FeG 4	FeD 4	TP 4	TD 4	FP 4	FD 4
Macropus spp.	SC 1	PA 1	UP 2	HD 3	RP 4	FeG 4	FeD 5	HP 6	FD 7	FeH 8	TD 8	TP 9	FP 9	UD 10	RD 11
Phascolarctos cinereus	SC 1	PA 1	HP 2	HD 2	UP 2	UD 2	RD 2	FeD 2	TP 2	TD 2	FP 2	FD 2	RP 3	FeH 3	FeG 3
Thylacinus cynocephalus	SC 1	PA 2	UP 3	RP 3	UD 4	RD 4	TD 4	FD 4	HD 5	FeG 5	TP 5	FP 5	HP 6	FeH 6	FeD 6
Sarcophilus harrisii	SC 1	PA 1	HD 2	UP 2	FP 2	HP 3	TD 3	FD 3	RP 4	TP 4	UD 5	RD 5	FeG 5	FeH 6	FeD 7
Dasyurus spp.	SC 1	PA 1	HD 2	UP 3	FeG 4	RP 5	FeD 6	TD 6	FP 6	FeH 7	FD 7	HP 8	UD 8	RD 8	TP 8
Perameles spp.	PA 1	SC 2	HD 3	RP 4	FeD 4	HP 5	UP 5	UD 5	RD 5	FeH 5	FeG 5	TP 5	TD 5	FP 5	FD 5
Isoodon spp.	SC 1	PA 1	HD 2	HP 3	UP 3	UD 3	RP 3	RD 3	FeH 3	FeG 3	FeD 3	TP 3	TD 3	FP 3	FD 3
Dasypus spp.	RP 1	SC 2	HD 2	PA 2	UP 3	TD 4	HP 5	UD 5	RD 5	FeH 5	FeG 5	FeD 5	TP 5	FP 5	FD 5
Myrmecophaga tridactyla	SC 1	PA 1	RP 2	TD 2	HD 3	UP 3	RD 3	FP 3	FD 3	HP 4	UD 4	FeH 4	FeG 4	TP 4	FeD 5
Heteroyrax brucei	HD 1	PA 1	SC 2	RP 2	FeH 3	FeG 3	UP 4	FeD 4	TD 4	FD 4	UD 5	HP 6	RD 6	TP 6	FP 6
Procavia capensis	SC 1	HD 1	RP 1	PA 1	TD 2	FD 3	UP 4	FeH 5	FeG 5	FeD 5	HP 6	UD 6	RD 6	TP 6	FP 6
Orycteropus afer	SC 1	HD 1	PA 1	RP 2	TD 3	UP 4	RD 4	FD 4	UD 5	TP 5	HP 6	FeH 6	FeG 6	FeD 6	FP 6
Tenrec ecaudatus	SC 1	HD 2	PA 2	RP 3	FeH 3	HP 4	UP 4	UD 4	RD 4	FeG 4	FeD 4	TP 4	TD 4	FP 4	FD 4
Erinaceus europaeus	HD 1	RP 1	SC 2	FeG 3	FeH 4	PA 5	UP 6	TD 6	FD 6	HP 7	UD 7	RD 7	FeD 7	TP 7	FP 7
Capreolus capreolus	SC 1	HD 1	RP 1	PA 1	TD 2	UP 3	FeH 3	FeG 3	FeD 4	HP 5	RD 5	TP 6	UD 7		
Equus spp.	SC 1	PA 1	HD 2	RP 3	TD 4	FeD 5	RD 6	UP 7	TP 7	HP 8	FeG 8	FeH 9			
Perodicticus potto	HD 1	SC 2	PA 3	FeG 3	FeH 4	UP 5	RP 6	TP 6	FD 7	TD 8	UD 9	FP 9	RD 10	FeD 10	HP 11
Chlorocebus aethiops	HD 1	SC 2	PA 2	UP 3	FeH 3	FeG 3	RP 4	FeD 4	TP 5	TD 5	FP 5	FD 5	HP 6	RD 6	UD 7
Macaca mulatta	HD 1	SC 2	PA 2	FeH 3	RP 4	FeG 4	UP 5	TD 6	FD 7	FP 8	TP 9	FeD 10	HP 11	UD 11	RD 12
Papio spp.	HD 1	SC 2	PA 3	UP 4	FeH 5	FeG 5	RP 6	TD 7	FD 8	FeD 9	TP 9	FP 9	RD 10	HP 11	UD 11
Pan troglodytes	SC 1	HD 1	UP 2	PA 2	RP 3	FeG 3	FeH 4	HP 5	TD 5	FD 5	UD *6*	RD 6	FeD 6	TP 6	FP 6
Saimiri spp.	SC 1	HD 1	PA 1	UP 2	FeG 2	FeH 3	RP 4	TD 4	FD 5	FeD 6	HP 7	RD 7	TP 7	FP 7	UD 8
Marmota marmota	HD 1	SC 2	PA 2	FeH 2	FeG 2	TD 2	RP 3	FD 3	UP 4	HP 5	FP 5	UD 6	RD 6	FeD 6	TP 6

Supplementary Table S2 cont.

Castor	HD	PA	SC	RP	UP	FeG	TD	FD	HP	FP	FeH	FeD	TP	UD	RD
fiber	1	2	3	4	5	6	7	8	9	9	10	10	10	11	11
Myocastor	PA	HD	SC	TD	RP	FeG	FeH	FP	FD	HP	UP	TP	FeD	UD	RD
coypus	1	2	3	4	5	5	6	7	7	8	8	8	9	10	10
Hydrochoerus	SC	HD	RP	PA	FeH	FeG	TD	FD	UP	HP	UD	RD	FeD	TP	FP
hydrochaeris	1	1	1	1	2	2	2	2	3	4	4	4	4	5	5
Dasyprocta	SC	HD	PA	TD	RP	FD	UP	FeG	UD	RD	FeH	FeD	FP	HP	TP
leporina	1	1	1	1	2	2	3	3	4	4	4	4	4	5	6
Lepus	HD	PA	SC	RP	UP	TD	FD	RD	UD	FeH	FeG	FeD	HP	TP	FP
europaeus	1	2	3	4	5	6	6	7	8	8	8	8	9	9	9
Phoca	PA	SC	HD	RP	FeH	FeG	FeD	HP	TP	UP	FP	UD	FD	RD	TD
vitulina	1	2	3	3	4	4	4	5	5	6	7	8	9	10	10
Ursus	HD	RP	PA	SC	UP	TD	FD	FeG	FP	UD	TP	RD	FeD	FeH	HP
arctos	1	2	2	3	4	5	5	6	6	7	7	8	8	9	10
Felis	SC	HD	PA	RP	UP	TD	FeH	FeG	FD	FP	HP	UD	RD	FeD	TP
silvestris	1	1	1	2	3	3	4	4	4	5	6	6	6	6	6

Supplementary Table S3. Sequences of growth plate closure. Low numbers denote early ossification. Letters are abbreviations for growth plates. FD: fibula, distal; FP: fibula, proximal; FeD: femur, distal; FeG: femur, greater trochanter; FeH: femur, head; HD: humerus, distal; HP: humerus, proximal; PA: pelvis, acetabulum; RD: radius, distal; RP: radius, proximal; SC: scapula, coracoid; TD: tibia, distal; TP: tibia, proximal; UD: ulna, distal; UP: ulna, proximal. Shadings denote anatomical regions: dark grey, wrist; light grey, ankle.

Tachyglossus aculeatus	UP 1	RP 1	SC 2	PA 2	FeD 2	FP 2	HD 3	HP 4	TP 4	FeH 5	FeG 5	UD 6	TD 6	FD 6	RD 7
Didelphis spp.	SC 1	PA 1	UP 2	HD 3	RP 4	TD 4	FP 4	HP 5	UD 5	RD 5	FeH 5	FeG 5	FeD 5	TP 5	FD 5
Dorcopsis muelleri	SC 1	PA 1	HD 2	UP 2	RP 3	HP 4	UD 4	RD 4	FeH 4	FeG 4	FeD 4	TP 4	TD 4	FP 4	FD 4
Macropus spp.	SC 1	PA 1	UP 2	HD 3	RP 4	FeG 4	FeD 5	HP 6	FD 7	FeH 8	TD 8	TP 9	FP 9	UD 10	RD 11
Phascolarctos cinereus	SC 1	PA 1	HP 2	HD 2	UP 2	UD 2	RD 2	FeD 2	TP 2	TD 2	FP 2	FD 2	RP 3	FeH 3	FeG 3
Thylacinus cynocephalus	SC 1	PA 2	UP 3	RP 3	UD 4	RD 4	TD 4	FD 4	HD 5	FeG 5	TP 5	FP 5	HP 6	FeH 6	FeD 6
Sarcophilus harrisii	SC 1	PA 1	HD 2	UP 2	FP 2	HP 3	TD 3	FD 3	RP 4	TP 4	UD 5	RD 5	FeG 5	FeH 6	FeD 7
Dasyurus spp.	SC 1	PA 1	HD 2	UP 3	FeG 4	RP 5	FeD 6	TD 6	FP 6	FeH 7	FD 7	HP 8	UD 8	RD 8	TP 8
Perameles spp.	PA 1	SC 2	HD 3	RP 4	FeD 4	HP 5	UP 5	UD 5	RD 5	FeH 5	FeG 5	TP 5	TD 5	FP 5	FD 5
Isoodon spp.	SC 1	PA 1	HD 2	HP 3	UP 3	UD 3	RP 3	RD 3	FeH 3	FeG 3	FeD 3	TP 3	TD 3	FP 3	FD 3
Dasypus spp.	RP 1	SC 2	HD 2	PA 2	UP 3	TD 4	HP 5	UD 5	RD 5	FeH 5	FeG 5	FeD 5	TP 5	FP 5	FD 5
Myrmecophaga tridactyla	SC 1	PA 1	RP 2	TD 2	HD 3	UP 3	RD 3	FP 3	FD 3	HP 4	UD 4	FeH 4	FeG 4	TP 4	FeD 5
Heteroyrax brucei	HD 1	PA 1	SC 2	RP 2	FeH 3	FeG 3	UP 4	FeD 4	TD 4	FD 4	UD 5	HP 6	RD 6	TP 6	FP 6
Procavia capensis	SC 1	HD 1	RP 1	PA 1	TD 2	FD 3	UP 4	FeH 5	FeG 5	FeD 5	HP 6	UD 6	RD 6	TP 6	FP 6
Orycteropus afer	SC 1	HD 1	PA 1	RP 2	TD 3	UP 4	RD 4	FD 4	UD 5	TP 5	HP 6	FeH 6	FeG 6	FeD 6	FP 6
Tenrec ecaudatus	SC 1	HD 2	PA 2	RP 3	FeH 3	HP 4	UP 4	UD 4	RD 4	FeG 4	FeD 4	TP 4	TD 4	FP 4	FD 4
Erinaceus europaeus	HD 1	RP 1	SC 2	FeG 3	FeH 4	PA 5	UP 6	TD 6	FD 6	HP 7	UD 7	RD 7	FeD 7	TP 7	FP 7
Capreolus capreolus	SC 1	HD 1	RP 1	PA 1	TD 2	UP 3	FeH 3	FeG 3	FeD 4	HP 5	RD 5	TP 6	UD 7		
Equus spp.	SC 1	PA 1	HD 2	RP 3	TD 4	FeD 5	RD 6	UP 7	TP 7	HP 8	FeG 8	FeH 9			
Perodicticus potto	HD 1	SC 2	PA 3	FeG 3	FeH 4	UP 5	RP 6	TP 6	FD 7	TD 8	UD 9	FP 9	RD 10	FeD 10	HP 11
Chlorocebus aethiops	HD 1	SC 2	PA 2	UP 3	FeH 3	FeG 3	RP 4	FeD 4	TP 5	TD 5	FP 5	FD 5	HP 6	RD 6	UD 7
Macaca mulatta	HD 1	SC 2	PA 2	FeH 3	RP 4	FeG 4	UP 5	TD 6	FD 7	FP 8	TP 9	FeD 10	HP 11	UD 11	RD 12
Papio spp.	HD 1	SC 2	PA 3	UP 4	FeH 5	FeG 5	RP 6	TD 7	FD 8	FeD 9	TP 9	FP 9	RD 10	HP 11	UD 11
Pan troglodytes	SC 1	HD 1	UP 2	PA 2	RP 3	FeG 3	FeH 4	HP 5	TD 5	FD 5	UD 6	RD 6	FeD 6	TP 6	FP 6
Saimiri spp.	SC 1	HD 1	PA 1	UP 2	FeG 2	FeH 3	RP 4	TD 4	FD 5	FeD 6	HP 7	RD 7	TP 7	FP 7	UD 8
Marmota marmota	HD 1	SC 2	PA 2	FeH 2	FeG 2	TD 2	RP 3	FD 3	UP 4	HP 5	FP 5	UD 6	RD 6	FeD 6	TP 6

Supplementary Table S2 cont.

Castor	HD	PA	SC	RP	UP	FeG	TD	FD	HP	FP	FeH	FeD	TP	UD	RD
fiber	1	2	3	4	5	6	7	8	9	9	10	10	10	11	11
Myocastor	PA	HD	SC	TD	RP	FeG	FeH	FP	FD	HP	UP	TP	FeD	UD	RD
coypus	1	2	3	4	5	5	6	7	7	8	8	8	9	10	10
Hydrochoerus	SC	HD	RP	PA	FeH	FeG	TD	FD	UP	HP	UD	RD	FeD	TP	FP
hydrochaeris	1	1	1	1	2	2	2	2	3	4	4	4	4	5	5
Dasyprocta	SC	HD	PA	TD	RP	FD	UP	FeG	UD	RD	FeH	FeD	FP	HP	TP
leporina	1	1	1	1	2	2	3	3	4	4	4	4	4	5	6
Lepus	HD	PA	SC	RP	UP	TD	FD	RD	UD	FeH	FeG	FeD	HP	TP	FP
europaeus	1	2	3	4	5	6	6	7	8	8	8	8	9	9	9
Phoca	PA	SC	HD	RP	FeH	FeG	FeD	HP	TP	UP	FP	UD	FD	RD	TD
vitulina	1	2	3	3	4	4	4	5	5	6	7	8	9	10	10
Ursus	HD	RP	PA	SC	UP	TD	FD	FeG	FP	UD	TP	RD	FeD	FeH	HP
arctos	1	2	2	3	4	5	5	6	6	7	7	8	8	9	10
Felis	SC	HD	PA	RP	UP	TD	FeH	FeG	FD	FP	HP	UD	RD	FeD	TP
silvestris	1	1	1	2	3	3	4	4	4	5	6	6	6	6	6

Supplementary Table S4. Summary of literature data about growth plate closure sequences of different taxa. n/a: not applicable; d: dry bone; r: radiography; ts: thin sections; * and references therein; A: epiphysis and diaphysis are fused by bone/are united; B: suture line absent; C: a fine line/defined portion of line is still visible.

Taxa	Common name	Authors	N	Method	Definition of fusion
Didelphimorphia					
Didelphis, *Philander* & *Metachirus*	Opossum	Washburn, 1964	30	d	A
Proboscidea					
Elephas maximus	Asian elephant	Roth, 1984	20	d	C
Loxodonta africana	African bush elephant	Roth, 1984	23	d	C
Sirenia					
Trichechus & *Dugong*	Manatee & Dugong	Todd & Todd, 1938	19	d	n/a
Rodentia					
Mus musculus (?)	Mouse	Dawson, 1935	n/a	ts	n/a
Rattus norvegicus (?)	Albino rat	Dawson, 1925, Dawson,1927	n/a	ts	n/a
Scandentia					
Tupaia glis	Common treeshrew	Shigehara, 1980	150	r	C
Primates					
Homo sapiens	Human	Stevenson, 1924	110	d	C
Homo sapiens	Human	Todd, 1930	<1000	d/r	C
Homo sapiens	Human	Scheuer et al., 2000	n/a	n/a	n/a
Homo sapiens	Human	Curgy, 1965*	n/a	n/a	n/a
Pongo spp.	Orangutan	Hofer et al., 1965*	n/a	n/a	n/a
Pan troglodytes	Chimpanzee	Hofer et al., 1965*	n/a	n/a	n/a
Gorilla gorilla	Gorilla	Randall, 1944	199	r	A
n/a	Gibbon	Hofer et al., 1965	n/a	n/a	n/a
Macaca fuscata	Japanese macaque	Kimura & Hamada, 1990	205	d	n/a
Macaca fuscata	Japanese macaque	Hayama, 1965	339	r	n/a
Macaca mulatta	Rhesus monkey	Cheverud, 1981	229	d	A
Macaca mulatta	Rhesus monkey	Cheverud, 1981*	n/a	n/a	n/a
n/a	Macaque and Langur	Hofer et al., 1965	n/a	n/a	n/a
Papio cynocephalus	Yellow baboon	Bramblet, 1969	60	d	n/a
Nasalis larvatus	Proboscis Monkey	Hofer et al., 1965*	n/a	n/a	n/a

Supplementary Table S4 cont.

Taxa	Common name	Authors	N	Method	Definition of fusion
n/a	Guenon and Mangabey	Hofer et al., 1965	n/a	n/a	n/a
Saguinus fuscicollis	Saddle-back tamarin	Glassman, 1983	84	d	B
Saguinus fuscicollis	Saddle-back tamarin	Kohn et al., 1997	36	d	A
Saguinus oedipus	Cottontop tamarin	Kohn et al., 1997	67	d	A
Saguinus nigricollis	Black-mantled tamarin	Tappen & Severson, 1971	31.00	d	A
Saimiri sciureus	Common squirrel monkey	Tappen & Severson, 1971	65.00	d	A
Cebus albifrons	White-fronted capuchin	Tappen & Severson, 1971	25.00	d	A
Callithrix jacchus	Common marmoset	Kohn et al., 1997	43	d	A
n/a	Marmoset	Hofer et al., 1965	n/a	n/a	n/a
n/a	Capuchin and Spider Monkey	Hofer et al., 1965	n/a	n/a	n/a
Lipotyphla					
Suncus murinus	Ryukyu house shrew	Shigehara, 1980	334	r	C
Artiodactyla					
Alces alces	Eurasian elk	Habermehl, 1985*	n/a	n/a	n/a
Dama dama	Fallow deer	Pohlmeyer, 1985	40	d	n/a
Odocoileus hemionus	Black-tailed deer	Lewall & Cowan, 1963	34	r	C
Odocoileus virginianus	White-tailed deer	Purdue, 1983	76	d	C
Bison bonasus	European bison	Koch, 1935	53	d	n/a
Capra ibex	Alpine ibex	Habermehl, 1985*	n/a	n/a	n/a
Capra ibex	Alpine ibex	Habemehl, 1992	9	d	n/a
Capra pyrenaica	Spanish ibex	Serrano et al., 2004	35	d	B
Ovis canadensis	Rocky Mountain bighorn sheep	Walker, 1987	51	d	B
Gazella gazella	Mountain gazelle	Davis, 1980	27	d	n/a
Sus scrofa	Wild boar	Bull & Payne, 1982	18	d	B
Phocoena phocoena	Harbour porpoise	Galatius & Kinze, 2003	350	d	B
Phocoena phocoena	Harbour porpoise	Galatius et al., 2006	158	r	C
Stenella coeruleoalba	Striped dolphin	Calzada & Aguilar, 1996	98	r	C
Carnivora					
Canis lupus	Wolf	Habermehl, 1985*	n/a	n/a	n/a
Vulpes vulpes	Red fox	Harris, 1978	28	d/r	n/a

Supplementary Table S5. Growth plate closure sequences of literature data. Postcranial events are ranked according to relative timing of growth plate closure. lu: lapsed (never completed) union; n/a: not applicable; * and references therein; FD: fibula, distal; FP: fibula, proximal; FeD: femur, distal; FeG: femur, greater trochanter; FeH: femur, head; HD: humerus, distal; HP: humerus, proximal; PA: pelvis, acetabulum; RD: radius, distal; RP: radius, proximal; SC: scapula, coracoid; TD: tibia, distal; TP: tibia, proximal; UD: ulna, distal; UP: ulna, proximal.

Taxa / gender	Authors	SC	HP	HD	UP	UD	RP	RD	PA	FeH	FeG	FeD	TP	TD	FP	FD
Didelphimorphia																
Didelphis, *Philander* & *Metachirus*	Washburn, 1964	1	6	3	2	8	5	7	1	11	8	10	10	9	10	9
Proboscidea																
Elephas maximus	Roth, 1984	n/a	6	1	2	8	n/a	n/a	n/a	9	7	4	5	3	n/a	n/a
Loxodonta africana	Roth, 1984	n/a	6	1	5	7	n/a	n/a	n/a	8	8	4	3	2	n/a	n/a
Sirenia																
Trichechus & *Dugong*	Todd & Todd, 1938	4	5	3	2	5	1	5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Rodentia																
Mus musculus (?)	Dawson, 1935	3	lu	1	lu	lu	2	lu	3	lu	lu	lu	lu	2	lu	4
Rattus norvegicus (?)	Dawson, 1925, Dawson,1927	5	11	1	7	10	2	10	3	8	8	9	9	3	10	4
Scandentia																
Tupaia glis, female	Shigehara, 1980	n/a	7	1	3	6	2	7	n/a	5	n/a	6	6	5	7	4
Tupaia glis, male	Shigehara, 1980	n/a	8	3	2	5	1	5	n/a	4	n/a	8	7	3	7	6
Primates																
Homo sapiens	Stevenson, 1924	2	8	1	4	7	5	7	3	5	5	7	7	6	7	6
Homo sapiens, female	Todd, 1930a	n/a	8	1	1	7	1	6	2	4	4	5	5	3	5	3
Homo sapiens, male	Todd, 1930a	n/a	8	1	2	7	2	7	3	5	5	6	6	4	6	4
Homo sapiens, female	Scheuer et al., 2000	8	6	2	2	8	1	7	2	4	6	8	6	6	5	3
Homo sapiens, male	Scheuer et al., 2000	3	7	1	1	8	2	7	2	4	5	7	5	4	6	4
Homo sapiens	Curgy, 1965*	2	10	2	3	6	4	7	1	6	5	9	7	5	8	6
Pongo spp.	Hofer et al., 1965*	na	10	1	2	12	4	11	na	3	4	9	8	6	7	5
Pan troglodytes	Hofer et al., 1965*	na	10	1	2	11	4	10	na	5	3	7	8	5	9	6
Gorilla gorilla	Randall, 1944	1	2	1	1	3	2	3	1	2	2	2	2	2	2	2
Gibbon	Hofer et al., 1965*	na	12	1	2	11	3	10	na	4	3	9	8	5	7	6
Macaca fuscata fuscata, female	Kimura & Hamada, 1990	3	7	1	6	6	6	6	2	6	4	6	6	6	6	6
Macaca fuscata fuscata, male	Kimura & Hamada, 1990	2	8	1	4	7	4	7	2	6	4	7	6	5	6	5
Macaca fuscata yakui, female	Kimura & Hamada, 1990	2	n/a	1	5	6	5	6	1	6	4	5	5	5	5	5

Supplementary Table S5 cont.

Taxa / gender	Authors	SC	HP	HD	UP	UD	RP	RD	PA	FeH	FeG	FeD	TP	TD	FP	FD
Macaca fuscata yakui, male	Kimura & Hamada, 1990	1	6	2	5	3	3	5	1	3	3	4	4	3	4	4
Macaca fuscata, female	Hayama, 1965	n/a	9	1	3	8	3	7	n/a	3	n/a	4	6	6	5	5
Macaca fuscata, male	Hayama, 1965	n/a	9	1	4	10	2	8	n/a	4	n/a	6	6	3	7	5
Macaca mulatta, female	Cheverud, 1981	n/a	8	1	3	10	5	9	2	4	5	8	6	6	6	7
Macaca mulatta, male	Cheverud, 1981	n/a	10	1	5	11	5	10	2	3	4	9	9	6	8	7
Macaca mulatta, female	Cheverud, 1981*	n/a	11	1	3	10	2	9	1	4	n/a	7	8	5	6	5
Macaca mulatta, male	Cheverud, 1981*	n/a	10	1	6	9	4	8	2	3	n/a	9	5	9	7	6
Macaque and Langur	Hofer et al., 1965*	n/a	12	1	2	9	3	10	n/a	4	5	11	10	6	8	7
Papio cynocephalus	Bramblet, 1969	2	7	1	3	5	3	5	2	5	4	6	5	4	5	4
Nasalis larvatus	Hofer et al., 1965*	n/a	12	1	2	10	4	11	n/a	4	3	9	7	5	8	6
Guenon and Mangabey	Hofer et al., 1965*	n/a	11	1	2	12	3	13	n/a	4	5	9	10	6	8	7
Saguinus fuscicollis	Glassman, 1983	3	5	1	4	5	4	5	2	4	4	7	6	4	6	4
Saguinus fuscicollis	Kohn et al., 1997	4	9	1	5	10	5	10	3	6	6	11	8	7	n/a	n/a
Saguinus oedipus	Kohn et al., 1997	3	7	1	4	10	5	8	2	4	4	11	9	6	n/a	n/a
Saguinus nigricollis	Tappen & Severson, 1971	3	13	1	5	10	6	11	2	7	4	12	13	8	12	9
Saimiri sciureus	Tappen & Severson, 1971	1	8	1	3	8	4	7	2	6	5	8	7	5	8	5
Cebus albifrons	Tappen & Severson, 1971	1	11	3	7	10	4	10	2	6	5	10	10	7	9	8
Callithrix jacchus	Kohn et al., 1997	2	9	1	4	9	4	8	2	6	5	8	7	4	n/a	n/a
Marmoset	Hofer et al., 1965*	n/a	12	1	4	9	2	8	n/a	3	5	13	11	7	10	6
Capuchin and Spider Monkey	Hofer et al., 1965*	n/a	10	1	2	12	4	11	n/a	4	3	7	8	5	9	6
Lipotyphla																
Suncus murinus riukiuanus, female	Shigehara, 1980	n/a	6	1	3	4	2	4	n/a	5	n/a	7	8	4	8	4
Suncus murinus riukiuanus, male	Shigehara, 1980	n/a	7	1	3	5	2	5	n/a	6	n/a	7	8	4	8	4
Artiodactyla																
Alces alces	Habermehl, 1985*	n/a	3	1	3	n/a	2	3	2	3	3	3	3	3	n/a	n/a
Dama dama	Pohlmeyer, 1985	3	12	4	7	10	1	10	2	8	6	8	11	5	n/a	n/a
Odocoileus hemionus, female	Lewall & Cowan, 1963	n/a	5	2	4	5	1	5	n/a	3	3	4	5	1	n/a	n/a
Odocoileus hemionus, male	Lewall & Cowan, 1963	n/a	4	1	3	4	1	3	n/a	3	3	4	3	2	n/a	n/a
Odocoileus virginianus, female	Purdue, 1983	n/a	6	3	4	4	1	4	2	5	5	4	4	3	n/a	n/a
Odocoileus virginianus, male	Purdue, 1983	n/a	8	3	7	6	1	7	2	7	7	7	7	4	n/a	n/a

Supplementary Table S5 cont.

Taxa / gender	Authors	SC	HP	HD	UP	UD	RP	RD	PA	FeH	FeG	FeD	TP	TD	FP	FD
Bison bonasus	Koch, 1935	1	9	2	5	8	4	8	10	6	6	7	7	3	7	6
Capra ibex	Habermehl, 1985*	n/a	n/a	n/a	n/a	4	n/a	4	1	2	3	3	6	3	n/a	n/a
Capra ibex	Habemehl, 1992	1	7	1	5	7	1	7	1	3	3	4	6	2	n/a	n/a
Capra pyrenaica	Serrano et al., 2004	2	10	3	7	7	1	5	3	8	6	8	9	4	n/a	n/a
Ovis canadensis, female	Walker, 1987	1	6	1	5	5	2	5	3	6	6	5	5	4	n/a	n/a
Ovis canadensis, male	Walker, 1987	1	3	1	3	3	1	3	1	4	4	3	3	2	n/a	n/a
Gazella gazella	Davis, 1980	2	5	1	5	n/a	1	5	n/a	4	4	4	5	3	n/a	n/a
Sus scrofa	Bull & Payne, 1982	1	4	2	4	3	1	4	1	3	3	4	5	2	4	3
Phocoena phocoena	Galatius & Kinze, 2003	n/a	2	1	1	3	1	3	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Phocoena phocoena, female	Galatius et al., 2006	n/a	n/a	1	1	3	2	4	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Phocoena phocoena, male	Galatius et al., 2006	n/a	n/a	1	2	4	3	5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Stenella coeruleoalba	Calzada & Aguilar, 1996	n/a	1	3	2	4	2	4	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Carnivora																
Canis lupus	Habermehl, 1985*	n/a	5	1	2	5	3	5	n/a	5	5	4	5	3	5	3
Vulpes vulpes	Harris, 1978	n/a	7	n/a	2	6	3	6	1	6	5	5	6	4	6	4

Supplementary Table S6. Marsupial species and genera investigated in this study and growth plates that show lapsed (never completed) union (lu). FD: fibula, distal; FP: fibula, proximal; FeD: femur, distal; FeG: femur, greater trochanter; FeH: femur, head; HD: humerus, distal; HP: humerus, proximal; PA: pelvis, acetabulum; RD: radius, distal; RP: radius, proximal; SC: scapula, coracoid; TD: tibia, distal; TP: tibia, proximal; UD: ulna, distal; UP: ulna, proximal.

	SC	HP	HD	UP	UD	RP	RD	PA	FeH	FeG	FeD	TP	PD	FP	FD
Macropus spp.							lu								
Dorcopsis spp.		lu			lu		lu		lu	lu	lu	lu	lu	lu	lu
Phascolarctos cinereus							lu								
Perameles spp.		lu		lu	lu		lu		lu	lu		lu	lu	lu	lu
Isoodon spp.		lu													
Thylacinus cynocephalus															
Sarcophilus harrisii											lu				
Dasyurus spp.		lu			lu		lu					lu			
Didelphis spp.		lu			lu		lu		lu	lu	lu	lu	lu		lu

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CHAPTER 2

No change in the sequence of dental, skeletal, and sexual maturity in dogs due to domestication

This work was done during the second and third year of my PhD. I collected the data with the help of KG and FL. I conducted the analyses and wrote the manuscript. All authors then participated in discussing and drafting the final manuscript.

This article is ready for submission.

No change in the sequence of dental, skeletal, and sexual maturity in dogs due to domestication

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Domestic dogs are descendants of the wolf (*Canis lupus*). It has been argued that the domestication process has altered the sequence of dental maturity, skeletal maturity, and sexual maturity due to altered environmental conditions. The aim of our study is to test this hypothesis by investigating a comprehensive sample of domestic dogs. We documented the timing of completed eruption of permanent dentition into occlusion as a proxy for dental maturity and the timing of growth plate closure at the proximal humerus as a proxy for skeletal maturity in cross-sectional ontogenetic series of wolves and 23 domestic dog breeds. Data for 225 domestic dog and 72 wolf individuals were collected based on radiographs and examination of macerated bones in veterinary facilities and museum collections. Our analyses show that there is no change in the sequence of dental, skeletal, and sexual maturity in dogs due to domestication and that, despite their great variability, domestic dogs are wolf-like in terms of the here investigated processes of somatic and sexual maturation.

Keywords: domestic dog, wolf, tooth, growth plate, eruption, closure

Introduction

Domestic dogs are descendants of the wolf (*Canis lupus*) [1-6]. When, where, and how the domestication process started is still a subject of debate [1,7,8]. Certainly, tame wolves in the custody and mastery of humans at the beginning of the domestication process experienced harsh changes of environmental conditions and hence changes in selective pressure, unrelated to any possible conscious and intentional selective pressures implemented by humans [2,9,10]. These changes might have included unpredictability of quantity, quality, and timing of food supplies, decreasing interspecific competition, and increasing intraspecific competition [2,9,10]. Tchernov and Horwitz [10] hypothesised that the uncertain environmental conditions during the early domestication process might have led to a cyclical reaction of accelerated maturation and increased reproductive capacity (larger litter sizes and short generation times). This would then have resulted in an lower age at sexual maturity and thus smaller body size in adults [10], latter being a feature which is characteristic for early stages of domestication in mammals [2,10-13]. Alternative and complementary explanations for the reduction in body size and increased fecundity in domesticated dogs are adaptation of the dogs to a new niche, created by permanent human settlements and human garbage, 'relaxed selection' due to protection and care by humans, conscious selection by humans for smaller individuals which are easier to control, and conscious selection by humans for larger litter sizes which leads – due to subsequent resource depletion – to smaller adult body size [14,15].

The body size diminution and accelerated

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maturation due to domestication have been hypothesised to be related to postnatal growth changes. Shigehara [16] compared the attainment of sexual maturity, dental maturity (completed emergence of the permanent dentition into occlusion), and skeletal maturity (closure of the growth plates in long bones) in domestic dogs and the wolf. Shigehara [16] proposed that domestic dogs attain sexual maturity earlier than skeletal and dental maturity, while wolves attain sexual maturity only after dental and skeletal maturity. The author further hypothesised that this heterochronic shift of sexual maturity is the result of the above mentioned changes in environmental conditions during early domestication, as described above. The early shift of sexual maturity in respect to dental and skeletal maturity would also be expected because uncertain nutritional supply during domestication (see above) does not so much affect the skeleton and teeth as it does sexual maturity [17]. However, Shigehara [16] based this hypothesis on only a few references and did not conduct more extensive studies. The goals of our study were therefore to investigate the sequence of dental, skeletal, and sexual maturity in domestic dogs versus wolves and for the first time test Shigehara's [16] hypothesis with a large and comprehensive dataset including a great variety of different domestic dog breeds.

Materials and Methods

Specimens

We sampled 225 domestic dog specimens representing 23 different breeds and 72 wolf specimens (*Canis lupus* and *Canis lupus chanco*) (Table 1 & 2): for the examination of the timing of dental maturity, dry skulls of 128 domestic dogs (19 breeds) and 47 wolves were used; for the examination of the timing of skeletal maturity, radiographs of 97 domestic dogs (12 breeds, radiographs) and dry bones of 25 wolves were used. We preferably sampled individuals up to two years of ages, since both wolves and dogs reach full maturity in this period of time [18-26]. We included both sexes because the influence of sex on growth plate closure was reported to be not significant [27-32], whereas only one study argued that there is some variation due to sex [33]. Any influence of sex on the timing of tooth eruption has not yet been reported. Specimens exhibiting pathologies or specimens for which pathology affecting limb bones or the skull was reported were not included. Specimens reported to have been neutered were not considered, since neutered dogs close their growth plates relatively late in comparison to untreated puppies [32].

Only specimens with reported individual age were used. In the case of the domestic dogs and captive wolves, the age data were sourced from databases populated by the corresponding pet owners and zoological gardens. Individual ages of the wild wolves were approximated using information provided by cementum annuli counts and known breeding seasons and gestation times (Supplementary information). Individual ages in all specimens in this study were usually assessed in days and subsequently converted to months.

Dry bones and skulls from the following institutions were examined to categorise status of dental eruption in domestic dogs and the wolf as well as of growth plate closure in the wolf: Naturhistorisches Museum Basel (NMB); Naturhistorisches Museum Bern (NMBE); Naturhistoriska Riksmuseet, Stockholm (NRM); Paläontologisches Institut und Museum der Universität Zürich (PIMUZ); Zoologisches Museum der Universität Zürich (ZMUZH). Radiographs from the Vetsuisse-Fakultät Bern (VSB, Departement für klinische Veterinärmedizin) were examined to assess the investigation of growth plate closure in domestic dogs. Radiographs had been taken for medical reasons with a computed radiography system (CR, Fuijifilm, Dielsdorf, Switzerland) in the course of medical diagnostic processes in accordance to standard hospital procedures, in standard positions [34]. Our investigations using these radiographs were

Table 1. Number (n) of investigated specimens of the wolf and domestic dog breeds used for the investigation of the timing of tooth eruption

Group	n
Basset hound	2
Beagle	3
Bernese mountain dog	17
Boxer	7
Chihuahua	11
Dachshund	4
English bulldog	1
Fox terrier	4
French bulldog	17
German shepherd	13
Golden retriever	6
Great Dane	9
Greyhound	2
Leonberger	5
Newfoundland	9
Pug	6
Slowensky tschuwatsch	1
St. Bernard	7
Standard poodle	4
Wolf	47

Table 2. Number (n) of investigated specimens of the wolf and domestic dog breeds used for the investigation of the timing of proximal humeral growth plate closure.

Group	n
Basset hound	1
Beagle	7
Bernese mountain dog	28
Chihuahua	6
Dachshund	11
English bulldog	9
French bulldog	23
Pekingese	1
Petit Basset griffon Vendéen	1
Shih tzu	5
Standard poodle	4
Welsh Corgi Pembroke	1
Wolf	25

thus retrospective. All examined specimens, their corresponding identification numbers, and the corresponding names of the institutions in which the specimens are housed, are given in File S1.

Data acquisition

Dental maturity was defined to be attained in a specimen if all permanent teeth (i3/3, c1/1, p4/4, m2/3) are fully erupted. The eruption stage of each tooth in the maxilla and the mandible was therefore coded as described by Tappen and Severson [35] (Fig. 1a, b): 1, unerupted, the tooth is not visible above the alveolus; 2, erupting, the tooth crown is visible above the alveolus, but not yet in occlusion; 3, erupted, the tooth is in the occlusal plane and the enamel-cementum junction is visible above the alveolus. The time that elapses among stages is variable and depends on the tooth locus. This is apparent on the wolf example. While stage 1 for all teeth lasts until about 3 months of age, stage 2 can last from 1 month (e.g., incisors) up to 3 months (e.g., canine teeth) (File S1).

The closure of various growth plates in the wolf was investigated and it was decided for proximal humeral growth plate closure as an indicator for skeletal maturity since this plate fused at last (File S1). Each growth plate was scored as either open or closed (Fig. 1c, d). Growth plates of dry bones (wolf sample) were considered closed if the outer surface of the growth plate was at least in part obliterated by bone [36]. Growth plates on radiographs (domestic dog sample) were considered closed if the radiolucent (black) line between epiphysis (or apophysis) and metaphysis was no longer visible at least at one position along the growth plate [32,37-39]. A discussion about the methods used here for determination of skeletal maturity is provided in the Supplementary information.

Literature data on the timing of sexual maturity in different domestic dog breeds were obtained from Johnston et al. [40]. Records from

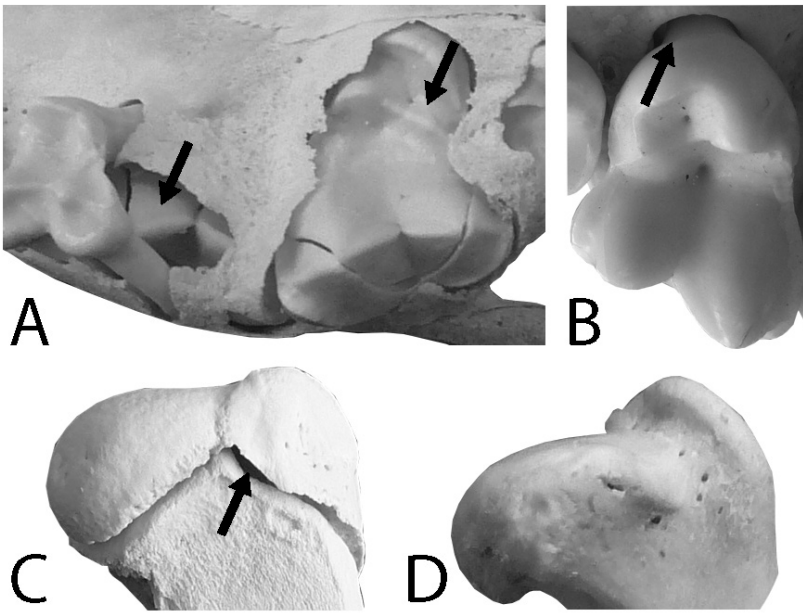

Figure 1. Growth plate closure stages (A,B,) and tooth eruption stages (C, D) as coded in this study. (A) left arrow, stage 1, unerupted, the tooth is not visible above the alveolus; right arrow, stage 2, erupting, the tooth crown is visible above the alveolus, but not yet in occlusion. (B) stage 3, erupted, the tooth is in the occlusal plane and the enamel-cementum junction (arrow) is visible above the alveolus. (C) stage 0, open growth plate (arrow). (D) stage 1, closed growth plate, outer surface is at least in part obliterated by bone (in this case, no growth line is visible any more).

wolves were obtained from various sources [41-44]. Extremely late records of first reproduction in in wolves (five or six years [26,45]) were not included. Only females were considered due to the better availability of data on sexual maturity.

Analyses

It has been debated whether the attainment of skeletal, dental, and sexual maturity in domestic dogs is associated with size differences and morphological disparity. A discussion and further analyses of these factors on our age estimates is given as Supplementary information. Since we could find no association of sexual maturity with body mass, this factor does not influence our age estimates of attainment of sexual maturity. However, chondrodystrophic breeds with disturbed cartilage and bone growth were not considered for further analyses in this study. Our sample size of domestic dogs for the estimation of the age at skeletal maturity thus comprises 77 specimens.

The age at completed emergence of permanent dentition into occlusion and closure of the proximal humeral growth plate was calculated as follows: mean value of the youngest and oldest ages of the specimens that had complete eruption/closure and incomplete eruption/closure, respectively. This procedure (or derivatives thereof) has been used in other studies concerned with timing and sequence of growth plate closure [46,47], although it implements a certain degree of error depending on the availability of specimens representing different individual ages. All analyses were conducted using past version 3 and Microsoft Excel 2010.

The age at (1) completed emergence of dentition into occlusion, (2) the age at closure of the proximal humeral growth plate, and (3) the age at attainment of sexual maturity in domestic dogs were compared to the estimates obtained from the wolf using non-parametric Mann-Whitney tests (equal variances $p > 0.05$ in all compared groups).

All analyses concerning the teeth were repeated twice, including and excluding the canine teeth. The complete eruption of the canine teeth is usually late and difficult to determine in some specimens because the alveolar bone around the canine teeth is prominent and obscures the enamel-cementum junction. Therefore, two separate calculations were made for the age at completed emergence of the permanent dentition into occlusion. One calculation excluded the canine teeth, taking into consideration only the incisors, premolars, and molars. The second calculation included the complete set of permanent teeth. Specimens in which the canine teeth were coded to be not completely erupted despite a relatively old age were not considered if they belonged to a breed where the masking of the enamel-cementum junction occurred regularly (French bulldog, Pug). Further, the Chihuahua was excluded from these age estimates because some specimens exhibited an extremely late shedding of deciduous teeth and complete eruption of the permanent teeth (File S1). Dental maturity as estimated excluding the canine teeth might be underestimated because the canine teeth are usually the latest teeth to erupt. On the other hand, dental maturity as estimated including the canine teeth might be overestimated due to the described obliteration of the enamel-cementum junction. We therefore consider both estimates as an age range within which the eruption is usually completed.

Results

The mean age at completed permanent tooth eruption into occlusion in domestic dogs is 5.5 months (sd = 0.5) excluding the canine teeth and 7.7 months (sd = 2.7) including the canine teeth. In wolves, the mean age at completed permanent tooth eruption into occlusion is 5.5 months (sd = 0.3) excluding the canine teeth and 7.3 months (sd = 0.1) including the canine teeth. The means of the domestic dogs and the wolf are not significantly different ($U = 2$, $p = 0.699$, including and excluding the canine teeth). The mean age at closure of the proximal humeral growth plate in non-chondrodystrophic domestic dogs is 10.5 months (sd = 0.2) and in wolves 11.4 months (sd = 0.5). The means of the domestic dogs and the wolf are not significantly different ($U = 0$, $p = 0.245$). The mean age of attainment of sexual maturity in the domestic dog is 12.5 months (sd = 3.4) and in the wolf 18.9 months (sd = 5.4). The means of the domestic dog and the wolf are not significantly different ($U = 36$, $p = 0.058$). The tooth eruption and growth plate closure stages for every specimen examined are given in File S1.

Discussion

Differences of dental, skeletal, and sexual maturity between domestic dog and wolf

The here estimated age of the attainment of dental maturity in domestic dogs (5.5 to 7.7 months) corresponds to previous findings by other authors of about 6 to 7 months in the German Shepherd [48] and 5.4 to 8.4 months in the bull terrier [49]. The here estimated age of attainment of dental maturity in wolves (5.5 to 7.3 months) is also in agreement with literature records reporting 6 to 7 months [26]. We found that the age at attainment of skeletal maturity in domestic dogs (10.3 to 10.7 months) lies in the range of previous findings from the literature (8.5 to 13.75 months) [27-29,31,33,37,50-53]. The here estimated attainment of skeletal maturity in the wolf (10.8 to 11.9 months) is also in agreement with the literature (10.8 to 12.3 months) [31]. The similarity of the age at attainment of skeletal maturity in the wolf and the domestic dog cannot reject previous hypotheses [54].

The variation of the attainment of the age at sexual maturity in the wolf is large. Most wild and captive wolves usually do not breed until the end of their second year or even much later [26,41-45]. However, captive female wolves were reported to show reproductive activity in their first year of life, at 9 – 10 months of age

[41,44]. Similarly early ages of first reproduction have been reported in a wild wolf population in which prey is abundant [26]. The attainment of sexual maturity in free living or straying female domestic dogs and dingoes, on the other hand, was reported to be variable but by trend younger than in wild wolves, with the earliest age at onset within the first year or at the age of one year [55-58], similar to most domestic dogs that are housed and fed by humans.

This difference in the attainment of sexual maturity between wolf and domestic dogs might be based on differences of the social structures in these two groups. In wolf-packs, only the dominant pair (alpha-female and alpha-male) reproduces. Sexual maturity in subordinate, pack members is usually supressed by the dominant female and young wolves mostly breed only after they disperse from their natal pack, usually at 11 – 24 months of age but sometimes also only in their fifth year [14,45,59]. In groups of straying and feral domestic dogs, on the other hand, all females reproduce and there is no controlling of reproduction of subordinate adults [56,60]. The early onset of reproduction in domestic dogs compared to wolves might thus be based on the more complex social system in wolves and a loss of susceptibility to social suppression of domestic dogs [41,61,62]. The loss of susceptibility to social suppression, in turn, has been speculated to be based on the close proximity of domestic dogs to humans and the subsequent ample opportunities to scavenge, which has made the dependence of domestic dogs to one another, and thus monogamy and true pack behaviour, unnecessary [61].

Sequence of dental, skeletal, and sexual maturity

Shigehara [16] hypothesised that in wild canids, dental maturity and skeletal maturity are attained before sexual maturity, with dental maturity preceding skeletal maturity. Thus, in wild canids, we expected to find the sequence dental – skeletal – sexual. In domestic dogs, on the other hand, Shigehara [16] hypothesised that this sequence is changed to sexual – dental – skeletal, most probably due to changed environmental conditions during domestication. Comparing the mean values, dental maturity is attained before skeletal maturity, and skeletal maturity is attained before sexual maturity in the wolf (dental – skeletal – sexual; Fig. 2). In domestic dogs, the comparison of mean values results in the same sequence as in the wolf (dental – skeletal – sexual), although the variation (sd) is larger and the sequence is not as clearly determinable as in the wolf (Fig. 2). Thus, we found that in the wolf, the sequence of dental, skeletal , and sexual is similar as speculated by Shigehara [16] (Fig. 2). In the domestic dog, however, we found no evidence for an early shift of sexual maturity compared to dental and skeletal maturity (Fig.2). Even though the age at attainment of sexual maturity in domestic dogs is on average earlier than in the wolf, which signifies a change in the direction as expected by Shigehara [16], the change is mild and does not affect the sequence of dental, skeletal, and sexual maturity. The similarity between domestic dogs and wolves in terms of dental, skeletal, and sexual maturity shows that the great changes of environmental conditions during early domestication [10] did not have a significant influence on these timings. Conservatism of life history variables within one species and among domestic breeds has also been reported by Clauss et al. [63]. To conclude, this study has shown that domestic dogs, although phenotypically extremely variable due to extensive artificial selection by humans, are still wolf-like in terms of the here investigated processes of somatic and sexual maturation.

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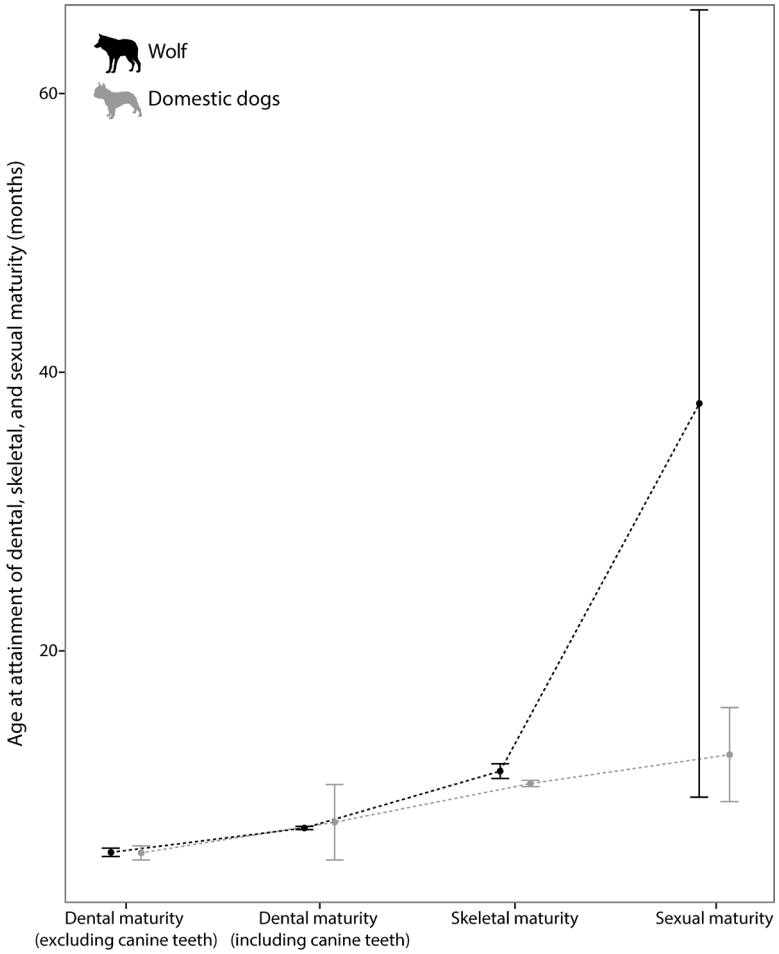

Figure 2. Summary of the ages at attainment of dental, skeletal, and sexual maturity in domestic dogs and the wolf. In both groups the sequence is dental – skeletal – sexual and no shift of sexual maturity in relation to dental and skeletal maturity in domestic dogs is observable. Dots represent mean age estimates and bars the associated standard deviations.

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Supplementary Information

Absolute age estimation of wild wolves

By default, the Naturhistoriska Riksmuseet (Stockholm) age estimates of wolves (in years) are based on cementum annuli counts [1]. We used these age statements in association with an approximated date of birth: wolves in Finland, at about the same latitude as the middle part of Sweden (about 60°N), where most Swedish wolves used in this study come from, were found to have breeding season in March [2,3]. Adding a gestation time of 63 days, which is the average in wolves [3-9], the birth of the puppies falls within May. According to the birth records of ten litters of captive wolves in Berne, Switzerland (47°N), the mean birth date is the 8th of May. Because the breeding season, and thus the date of whelping, gets later with increasing latitude [2,3], the average birth date of the Swedish wolves is probably slightly later than that observed in Swiss wolves. Thus, for the sake of simplicity, we suggest the 15th of May as the average birth date of the Swedish wolves. The age of the wolves from Naturhistoriska Riksmuseet was thus estimated as the cementum annuli count plus the difference between the 15th of May and their date of death. This approach implies an error of plus/minus about a half a month for calculations of completed permanent dentition into occlusion as well as of closure of the proximal humeral growth plate.

Discussion of data acquisition on growth plate closure in this study

There are two limitations concerning the study of growth plate closure as exerted here. First, we compare age estimates at the attainment of skeletal maturity using dry bones (wolf) and radiographs (domestic dogs). In their study about tooth eruption and growth plate fusion in pigs and wild boar, Bull and Payne [10] mentioned that it is not clear whether data about growth plate closure obtained through observing dry bones and radiographs are comparable. On the other hand, Harris [11] reported that growth plate closure in red foxes recorded by using radiographs and by examining dry bones yielded the same results.

The main problem in comparing data from dry bones and radiographs is the pattern of growth plate closure: the ossification starts in the central parts of the growth plate and subsequently extends to the outer surface [12]. If the central part of the growth plate starts to ossify, these parts of the growth plate appear as radiopaque areas in a radiograph. Such a growth plate would be considered as closed if the definition of growth plate closure used in this study is applied. We chose this definition because the bone does not grow any longer in length as soon as parts of the growth plate are ossified [13]. The definition of growth plate closure in dry bones used in this study is based on the obliteration of the outer surface of the bone. We chose this definition because in dry bones beginning closure in the centre cannot be determined. Very often it cannot be distinguished if an epiphysis or an apophysis is just glued to the metaphysis or actually already slightly ossified. Therefore, the age at closure of the growth plates using dry bones might be overestimated compared to estimates using radiographs. However, since our age estimations in the wolf and in the domestic dogs are close to the estimates from the literature (see Discussion) we conclude that the combination of radiographs and dry bones does not pose a problem in this study. The second limitation concerning the study of growth plate closure as exerted here applies to the radiographs used for this study. Since most radiographs were taken during standard diagnostic procedures, they often display left lateral views of the thoracic region. Due to the thoracic focus of these images, the humerus is on the outer rim and additionally superimposed with the contralateral humerus and possible other items, such as endotracheal tubes. In cases where the stage of closure was not determinable without doubt, the radiograph in question was therefore double checked and re-evaluated by more

than one of the authors. Additionally, there is one shortcoming of radiographs, which is that variability in energy and exposure time can result in structures being either undetected or overexposed and thus not visible on the radiograph.

Variation of dental, skeletal, and sexual maturity within domestic dogs

Within domestic dogs it has been disagreement as to whether there are breed specific differences of the age attainment of dental, skeletal, and sexual maturity. Many authors have reported breed specific differences of the timing of tooth eruption: Scott and Fuller [14] reported delayed completed eruption of the upper deciduous canine teeth in the fox terrier compared to basenji, beagle, cocker Spaniel, and Shetland sheepdog. Huidekoper [15] noted that the permanent teeth erupt several months earlier in large breeds compared to smaller terriers. Medium sized breeds (e.g., setters) are in an intermediate position. Similar statements were made by other authors [16-19]. However, none of these authors presented data to support these statements. Evans [20] went even further, stating that the life span of a breed correlates with the timing of tooth eruption: short lived, large breeds' teeth erupt earlier than long lived, small breeds' teeth. Similar statements have also been provided by several authors as described in Arnall [21].The sequence of growth plate closure has been reported to be constant among domestic dog breeds [22], but whether the absolute timing of closure of the growth plates is different among domestic dog breeds has been a matter of debate. Sumner-Smith [23] found that there are no differences among poodle, greyhound, and German shepherd, whereas Hare [22,24] reported differences of the timing of growth plate closure among breeds. Some degree of variation has been reported among specimens of the same breed [22,25-27] and even among litter mates in the red fox [11], the wolf [28], and the domestic dog [22,23]. Age at growth plate closure is further expected to depended on nutritional conditions [29] and age at time of neutering of puppies [30]. The small standard deviations (except for the completion of tooth eruption into the occlusal plane when canines are included) of our mixed-breed sample (see Results) suggest that breed specific differences and environmental influences are not greatly affecting our age estimates of dental and skeletal maturity.

It has further been discussed whether the age at attainment of skeletal maturity is associated with chondrodystrophy [22,31-33]. Chondrodystrophy is a congenital disturbance which affects mainly the cartilage and has a negative impact on the growth of endochondral bone, resulting in disproportionately short, thickened, and curved long bones [25,34,35]. Our sample contains six chondrodystrophic breeds as determined by Parker et al. [36] (basset hound, Dachshund, Pekingese, petit Basset griffon Vendéen, shih tzu, and Welsh corgi prembroke). It was tested here whether the attainment of skeletal maturity in the chondrodystrophic breeds (mean = 8.5, standard deviation (sd) = 0.02) is different from

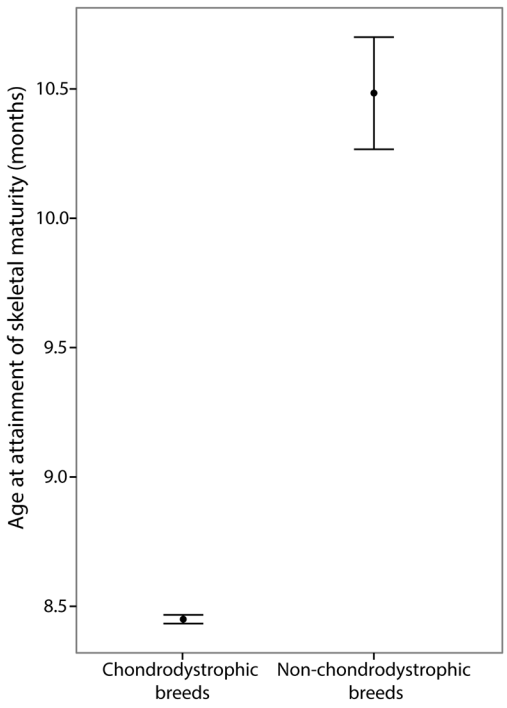

Figure S1. Age at attainment of skeletal maturity in chondrodystrophic and non-chondrodystrophic domestic dogs.

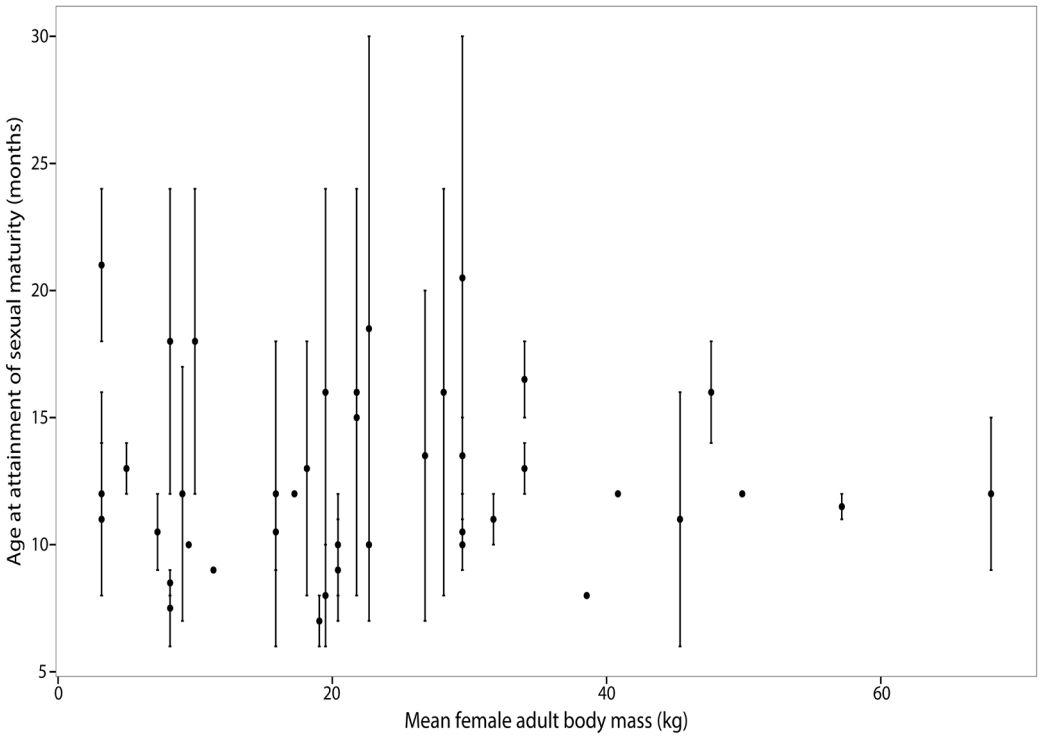

Figure S2. Age at attainment of sexual maturity in different domestic dog breeds exhibiting different body sizes. Data obtained from Johnston et al. [40].

non-chondrodystrophic breeds (mean = 10.5, sd = 0.22) using a non-parametric Mann-Whitney test (equal variances, F = 169, p = 0.098). We found that there is a marked difference in closure times between the two groups (Fig. S1), but one that this is not statistically significant (U = 0, p = 0.245). However, we only included non-brachycephalic breeds for all comparisons of with the wolf sample because the marked difference between non-chondrodystrophic and chondrodystrophic groups gives evidence for existing differences.

Among breeds of domestic dogs, females of small breeds are supposed to attain sexual maturity earlier than females of larger breeds [37-42], although this correlation has also been debated [41]. The age at sexual maturity may also be affected by line and breed dependent genetic factors, cross-breeding, diet, and housing conditions [37,38]. Records of the mean age at attainment of sexual maturity and adult female body weight in 43 domestic dogs breeds, ranging from around 3 kg (Affenpinscher) to around 68 kg (St. Bernard) were obtained from Johnston et al. [41]. A non-parametric Spearman's correlation was calculated and we found a non-significant correlation between mean adult female body mass and age at attainment of sexual maturity (rS = 0.064, p = 0.682) (Fig. S2).

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CHAPTER 3

Cranial suture closure in domestic dog breeds and relations to skull anatomy – a comparison with human patterns

This work was done during the second and third year of my PhD. SH and I collected the data. I conducted the analyses and wrote the manuscript. Both authors then participated in drafting the final manuscript.

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Cranial suture closure in domestic dog breeds and relations to skull anatomy – a comparison with human patterns

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Brachycephalic domestic dog breeds are characterised by a relatively short and broad skull with a dorsally rotated rostrum (airorhynchy). This skull conformation, together with accompanying peculiarities of the braincase, face, and teeth, resemble human syndromes associated with premature fusion of certain craniofacial sutures and cranial base synchondroses, e.g., achondroplasia, Apert syndrome, and Crouzon syndrome. In domestic dogs, the association of brachycephaly with patterns of suture and synchondroses closure is not well investigated. We aim to explore breed-specific patterns of cranial suture and synchondrosis closure in relation to the prebasial angle (proxy for airorhynchy) and hence brachycephaly in domestic dogs. For this purpose, we coded closure of 19 sutures and synchondroses in 30 wolves – the ancestor of all domestic dogs – and 147 domestic dogs comprising 12 breeds. Comparisons of the relative amount of closing and closed sutures and synchondroses (closure scores) in adult individuals showed that airorhynch breeds have significantly higher closure scores than non-airorhynch breeds and that domestic dogs have significantly higher closure scores than the wolf. In accordance with studies on humans, mice, and rabbits we further found that the prebasial angle is significantly positively correlated with the amount of closure of the fronto-parietal suture and the basispheno-presphenoid synchondrosis. These results indicate that similar mechanisms might act on the generation of the bulldog-type skull conformations in humans and domestic dogs. Further, also some sutures of the rostrum are significantly positively correlated with the prebasial angle, suggesting biomechanical influences on patterns of suture closure in domestic dogs.

Keywords: human, wolf, synchondroses, fusion, brachycephaly

Introduction

One of the most striking peculiarities of domestic dogs is their disparity of skull shape. Much of this disparity among breeds can be characterised by variation in the relative length of the rostrum and breadth of the skull as well as by the angulation of the rostrum relative to the cranial base (Rosenberg, 1966; Lüps, 1974; Nussbaumer, 1982; Brehm et al., 1985; Herre and Röhrs, 1990; Evans, 1993; Drake and Klingenberg, 2010). Traditionally, domestic dogs have been subdivided accordingly into ´short-head´ (brachycephalic, relatively short rostrum), ´long-head´ (dolichocephalic, relatively long rostrum), and ´medium-head´ (mesocephalic, intermediate between brachy- and dolichocephalic) breeds. Different classification schemes based on linear and angle measurements, as well as on qualitative characteristics (presence of temporal line, roundness of the cranial vault), have been developed in different fields of research (Brehm et al., 1985; Evans, 1993; Regodón et al., 1993; Koch et al., 2012). Table 1 lists several dog breeds and their corresponding allocation to the different skull proportion categories. Brachycephaly – the bulldog-like skull type – is most derived and characterised by a set of peculiarities, the most profound of which include relatively wide zygomatic arches, a relatively short rostrum, and airorhynchy (Table 2). The angulation of the rostrum at the plane of the palate relative to the

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cranial base (prebasial angle) is slightly declined (klinorhynch) in the wolf (*Canis lupus*) – the ancestor of all domestic dogs –, in other wild canids, e.g., jackal, and in domestic dogs phenotypically resembling wild canids, e.g., German shepherd (Nussbaumer, 1982; Herre and Röhrs, 1990). Thus, the rostrum is rotated ventrally with respect to the cranial base in most canids. However, bulldog-type brachycephalic domestic dog breeds exhibit a dorsal rotation of the rostrum relative to the cranial base. In these cases, the prebasial angle approaches 180° (orthocranial conformation) and even exceeds 180°, a condition called airorhynchy (Rosenberg, 1966; Nussbaumer, 1982).

Table 1. Domestic dog breeds and the wolf and their corresponding attribution to skull types. Groups are ordered according to ascending shoulder height, with the smallest breed on the top of the table. B, brachycephalic; D, dolichocephalic; M, or mesocephalic according to Brehm et al., (1985) and Koch et al., (2012). Brachycephalic breeds are highlighted in grey. Asterisks indicate brachycephalic breeds which are considered brachycephalic according to linear skull measurements but are not airorhynch according to Nussbaumer (1982) and this study.

Group	Group mean shoulder height (cm)	Skull type
Chihuahua	19.0	B*
Maltese	22.3	B*
Dachshund	23.5	M
French bulldog	30.0	B
Pekingese	37.7	B
Cocker spaniel	39.5	M
Medium Schnauzer	47.0	M
Chow Chow	51.0	M
Bull terrier	56.0	M
Boxer	58.3	B
German shepherd	60.0	M
Collie	61.0	D
Bernese mountain dog	64.8	M
Giant Schnauzer	65.3	M
St. Bernard dog	65.5	M
Dobermann pinscher	66.0	D
Afghan hound	66.6	D
Great Dane	78.5	M
Irish wolfhound	80.5	D

Skull shape is generated during prenatal development as well as during postnatal growth. Skull proportions, e.g., relatively short rostrum and wide skull in brachycephalic breeds, are already expressed in neonates as Starck (1962) described on the basis of comprehensive material of German shepherd, barsoi, whippet, Pekingese, Belgian dwarfed griffon, pug, French bulldog, bullterrier, dachshund, Bedlington terrier, greyhound, and Afghan hound. Therefore, the expression of brachycephalic skull proportions is already established during prenatal ontogeny. Airorhynchy, on the other hand, is established only during postnatal ontogeny. At birth, all domestic dogs are klinorhynch and breeds which usually exhibit airorhynchy as adults develop their enlarged prebasial angle throughout postnatal growth (Starck, 1962; Rosenberg, 1966). Important sites of skull growth during postnatal ontogeny are sutures and synchondroses. Sutures are fibrous joints between the dermal bones, or between the dermal and endochondral bones, in the vertebrate skull (Herring, 2000; Hall, 2005; Rice, 2008b). They are distinct from the cartilaginous synchondroses, which connect the endochondral bones of the cranial base (Cohen, 2000; Herring, 2000; Hall, 2005; Cendekiawan et al., 2010), albeit they are sometimes not considered separately and addressed as sutures, e.g., in Krogman (1930). The function of cranial sutures and synchondroses is threefold (Cohen, 2000; Cohen, 2008; Rice, 2008b): articulation and holding together the bony elements of the skull while allowing deformations (especially during birth), mechanical stress absorption, and bone growth. In sutures, growth of the adjacent bones occur by intramembranous osteogenesis at their m argins (Rice, 2008b). As distinct from sutures, the bones adjacent to synchondroses grow via endochondral ossification, similar to the growth plates of long bones (Hall, 2005; Nie, 2005; Cendekiawan

Table 2. Skull characteristics of brachycephalic domestic dog breeds. After Stockard (1941), Brehm et al., (1985), Evans (1993), Turner (2008), Martin (2009), and Schoenebeck and Ostrander (2013).

Characteristics of brachycephalic domestic dog breeds
Widened skull with wide zygomatic arches
Spherical skull vault [1]
Rounded and protruding forehead [1]
Increased skull height
Two temporal lines instead of a sagittal crest [1,2]
Length reduction of the bones of the rostrum and brachygnathic condition of the upper jaw
Protruding mandible and prognathic condition of the lower jaw [2]
Shallow orbits, ocular proptosis, and widely placed orbits [2]
Up-turned nasal tip [2]
Flat or convex (rather than concave) oral surface of the palate
Short basicranium
Persistent ventral foramen magnum [2]
Wide spread of incisors
Crowded premolars
Marked angle between molar and premolar region

[1] Probably a consequence of small body size in many brachycephalic breeds (Klatt, 1913).
[2] Not always observed and not in all breeds

et al., 2010). The fusion of one suture or synchondrosis stops any further growth at that location (Opperman, 2000; Rice, 2008b). There is a species-specific pattern after which sutures and synchondroses close and in which sequence (Rager et al., 2014).

Premature fusion of cranial sutures (craniosynostosis) is typically associated with an abnormal craniofacial shape due to a compensatory growth in patent sutures and trough bone remodelling (Morriss-Kay and Wilkie, 2005; Richtsmeier and Flaherty, 2013). A bilateral, premature fusion of the fronto-parietal (coronal) suture, for example, can lead to brachycephaly in humans, a condition that is characterised by a roundish cranial vault which is almost as long as wide (Cohen and MacLean, 2000; Rice, 2008a; Richtsmeier and Flaherty, 2013). Likewise, abnormalities in the growth of the cranial base are often accompanied by midfacial deficiencies due to the direct connection between facial bones and the cranial base as well as their integration into one growth complex in humans (Stockard, 1941; Nie, 2005; Schoenebeck and Ostrander, 2013). These midface deficiencies include short and depressed nasal bones, short bones of the upper jaw, cheekbones, and eye sockets (summarised as midface hypoplasia), and can result in mandibular prognathism. In humans, some syndromes that are associated with the above described facial and cranial peculiarities include premature fusion of the cranial base synchondroses and a reduced cranial base length, for example in achondroplasia. Some human syndromes are additionally associated with premature fusion of craniofacial sutures, especially the fronto-parietal suture, e.g., in Apert syndrome and Pfeiffer syndrome (Morriss-Kay and Wilkie, 2005; Nie, 2005; Rice, 2008a; Cendekiawan et al., 2010). Most syndromes involving premature closure of sutures and synchondroses, including the above mentioned ones, are caused by mutations in genes coding for fibroblast growth factor receptors (FGFR1, FGFR2, FGFR3) in humans (Hehr and Muenke, 1999; Morriss-Kay and Wilkie, 2005; Nie, 2005; Richtsmeier and Flaherty, 2013; Schoenebeck and Ostrander, 2013). These FGFRs belong to one of the five most important gene families involved in the patterning and morphogenesis of the skull and brain, whereby they play major roles in a multitude of fundamental cellular processes in many tissues (Horton et al., 2007; Richtsmeier and Flaherty, 2013).

These syndromes are not confined to humans. Studies have shown that achondroplasia causes similar skull features in mice and rabbits as it does in humans, including a shortened cranial base, shortened nasal bones, an increased size of the neurocranium, frontal bossing, a dome-shaped cranial vault, and a prognathic mandible (Brown and Pearce, 1945; Wadler Bloom et al., 2006). Moreover, it has been shown that mice with mutated

FGFR2 show craniofacial features similar to humans with Apert and Pfeiffer syndromes. These features include premature ossification of the fronto-parietal suture, jugo-squamosal suture (at the zygomatic arch), and premaxillo-maxillary suture, a shortened snout, a rounded skull, and shallow orbits, leading to ocular proptosis (eyes protrude out of their normal position) (Hajihosseini et al., 2001; Hajihosseini, 2008). In addition to that, experimentally induced premature closure of multiple cranial sutures in rabbits resulted in a dorsal rotation of the face relative to the cranial base (Babler and Persing, 1982; Babler, 1988).

The above mentioned features of the face, the neurocranium, and the teeth in some human syndromes resemble the facial characteristics of brachycephalic breeds of dogs (Stockard, 1941) (Fig. 1). Amongst these characteristics are relative shortness of the facial bones, prognathic mandible, a protruding forehead, shallow orbits, a short basicranium, and increased skull height (Table 2). Causal relationships between premature fusion of sutures and synchondroses to these peculiar features have so far been described for humans (Cohen et al., 1984; Cohen and MacLean, 2000; Celenk et al., 2003; Horton et al., 2007; Rice, 2008a; Richtsmeier and Flaherty, 2013), rabbits (Babler and Persing, 1982; Babler, 1988), and mice (Hajihosseini et al., 2001; Hajihosseini, 2008) but are not well known in domestic dogs. Moreover, the underlying mechanisms and genetic pathways are probably not the same as in humans, although similarities do exist (Parker et al., 2009; Schoenebeck and Ostrander, 2013). Yet, the short rostrum and bulged forehead in bulldog-like, brachycephalic types of dogs has been linked and directly compared to the craniofacial features and their causes in human achondroplasia (Stockard, 1941; Jezyk, 1985). Supporting this point of view is a recent study by Schmidt et al., (2013). They could show that the probability of finding a closed spheno-occipital synchondrosis at a given age is significantly higher in brachycephalic domestic dog breeds compared to mesocephalic ones, as in humans with achondroplasia. This has also been suggested by Schoenebeck and Ostrander (2013). Nevertheless, brachycephalic domestic dogs should not be considered canine equivalents of Apert or Pfeiffer human patients.

Despite the growing knowledge of genes which govern the vast phenotypic disparity of different domestic dog breeds (Fondon and Garner, 2004; Boyko et al., 2010; Schoenebeck et al., 2012; Schoenebeck and Ostrander, 2013), not much is known about the processes of development and growth on which the genes are acting and if postnatal growth is altered in the different skull types. Specifically, it has – to our knowledge – never been investigated how patterns of closure of the craniofacial sutures, which have been found to be related to a bulldog-like skull anatomy in humans, mice and rabbits (fronto-parietal, jugo-squamosal, premaxillo-maxillary), are

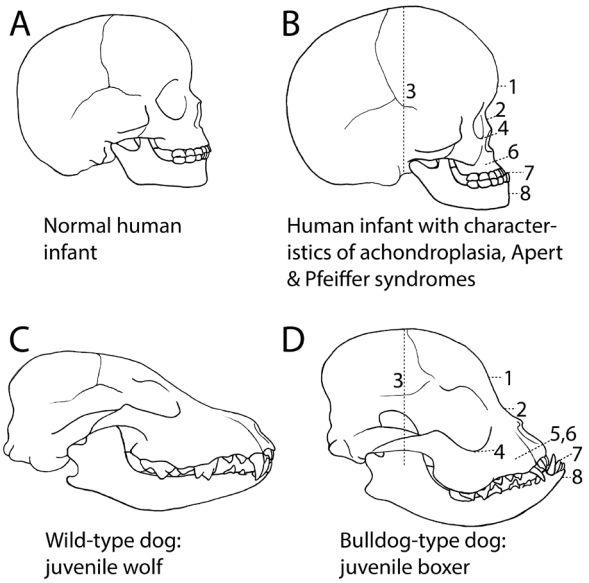

Figure. 1. Schematic illustrations of anatomical features of the skull and the teeth (1 – 8) which can occur in some human syndromes associated with premature fusion of certain cranial sutures and cranial base synchondroses (e.g., achondroplasia, Apert, and Pfeiffer syndromes) (B) as well as in bulldog-type domestic dog breeds (D), as compared to a normal human (A) and a mesocephalic canid (C). 1, frontal bossing; 2, shortened & depressed nasals; 3, increased skull height; 4, shallow orbits; 5, dorsal rotation of rostrum; 6, shortened maxilla; 7, dental malocclusion; 8, mandibular prognathism.

related to brachycephaly in different breeds of domestic dogs. Moreover, other cranio-facial sutures of the, or adjacent to, the rostrum are worth being considered in this respect because they are directly involved in a shortening and a dorsal rotation of the bones of the rostrum. This study aims at investigating patterns of cranial suture and synchondroses closure in domestic dog breeds with different skull conformations to find out if and how they are related to the brachycephalic phenotype.

Material and Methods

Data acquisition

Patterns of cranial suture closure were assessed by examining ontogenetic series of dry skulls of 30 wolves and 147 domestic dogs comprising 12 modern breeds (Table 3), which exhibit different skull conformations (Fig. 2, Table 3). Nineteen sutures and synchondroses of the neuro- and viscerocranium and the cranial base were coded, representing four functional groups according to the categorisation of Krogman (1930), which has been widely used in more recent studies (Wilson and Sánchez-Villagra, 2009; Goswami et al., 2013; Rager et al., 2014) (Table 4, Fig. 3): the basal group (cartilaginous cranial base synchondroses), the palatal group (sutures of the rostrum in ventral aspect), the facial group (sutures of the rostrum, periocular area, and cheeks in dorsal or lateral aspects), and the cranio-facial group (sutures which connect the face to the neurocranium). Additionally, the fronto-parietal suture was coded. These sutures were chosen due to their position between and adjacent to the bones of the rostrum and due to their known influence on cranial anatomy when closed prematurely in humans, mice, and rabbits (see Introduction). Persisting open fontanelles in some Chihuahua specimens were not considered.

For all analyses, only specimens ranging between 1 and 11 years of age were used. This age range was chosen because almost none of the coded sutures in any of the breeds examined and the wolf starts to obliterate in the first year of life while the whole skeleton is still growing. The upper age limit was chosen because all breeds examined and the wolf are represented by specimens in their eleventh year, older individuals were increasingly scarce in some groups. This procedure moreover ensures that comparable age classes and thus degrees of suture closure were compared. Further, the sampling was deliberately biased as to obtain an evenly distributed sample concerning the absolute individual ages and to include specimens which are historically as young as possible. The second point was important because some domestic dog breeds underwent significant changes due to breeding practices throughout the last century (Nussbaumer, 1982; Drake and Klingenberg, 2008). Further, only specimens with closure states applicable for all investigated sutures and synchondroses were used. Both sexes were considered.

Table 3. Cranial sutures and synchondroses investigated in this study and their allocation to Krogman's (1930) functional groups. F, frontal; l, lateral; v, ventral apects. No., number of suture.

No.	Suture/Synchondrosis	Functional group
1	Fronto-parietal	Vault
2	Basispheno-presphenoid	Cranial base
3	Basispheno-basioccipital	Cranial base
4	Exoccipito-supraoccipital	Cranial base
5	Exoccipito-basioccipital	Cranial base
6	Interpremaxillary (v)	Palatal
7	Intermaxillary	Palatal
8	Premaxillo-maxillary (v)	Palatal
9	Interpalatine	Palatal
10	Maxillo-palatine (v)	Palatal
11	Pterygo-palate (v)	Palatal
12	Premaxillo-maxillary (f)	Facial
13	Premaxillo-nasal	Facial
14	Internasal	Facial
15	Maxillo-jugal (l)	Facial
16	Maxillo-nasal	Facial
17	Naso-frontal	Cranio-facial
18	Lacrimo-frontal	Cranio-facial
19	Jugo-squamosal (l)	Cranio-facial

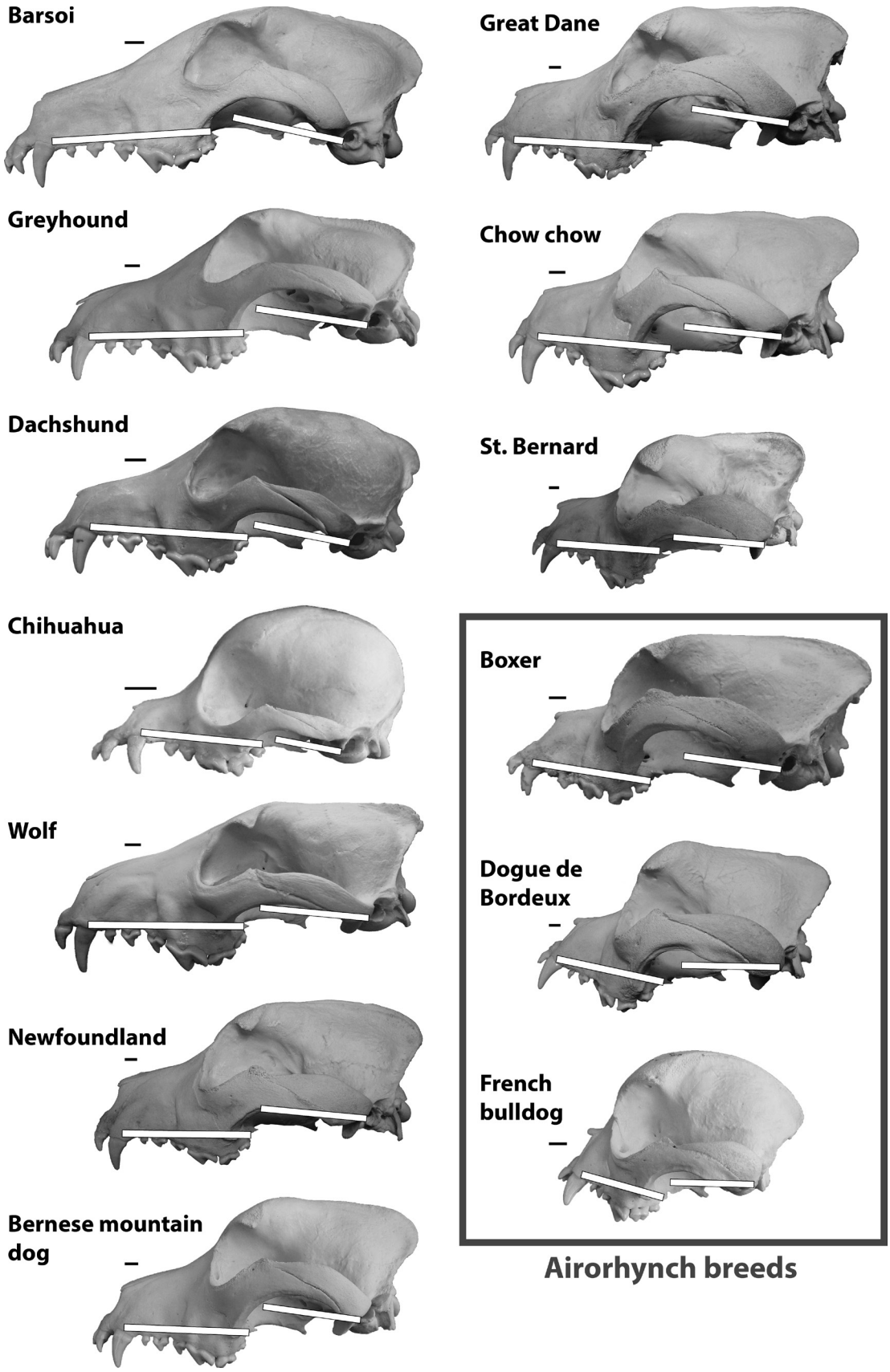

Figure 2. Skulls of the here investigated domestic dog breeds and the wolf in lateral aspect. The prebasial angles (angle between the hard palate and the cranial base of the skull) are indicated with white bars and the breeds are sorted from top to bottom and left to right according to increasing prebasial angles, i.e. increasing dorsal rotation of the rostrum (Table 4). Airorhynch breeds are indicated with a grey frame. Skulls are scaled to the same height of the skull. Scale bars are equal to 1 cm.

Each suture and synchondrosis was examined externally (Wilson and Sánchez-Villagra, 2009; Sánchez-Villagra, 2010; Goswami et al., 2013; Rager et al., 2014; Wilson, 2014) and the closure state was coded as depicted in Fig. 4: 0, open, the suture is completely open (Fig. 4a); 1, closing, parts of the suture are obliterated (Fig. 4b); 2, closed, the entire suture is obliterated and not discernible from the surrounding bone (Fig. 4c). The skulls were obtained from the following institutions (alphabetical order): Albert-Heim-Stiftung in the Naturhistorisches Museum Bern, Switzerland; Naturhistoriska riksmuseet, Stockholm, Sweden; Paläontologisches Institut und Museum der Universität Zürich, Switzerland; Zoologisches Museum der Universität Zürich, Switzerland.

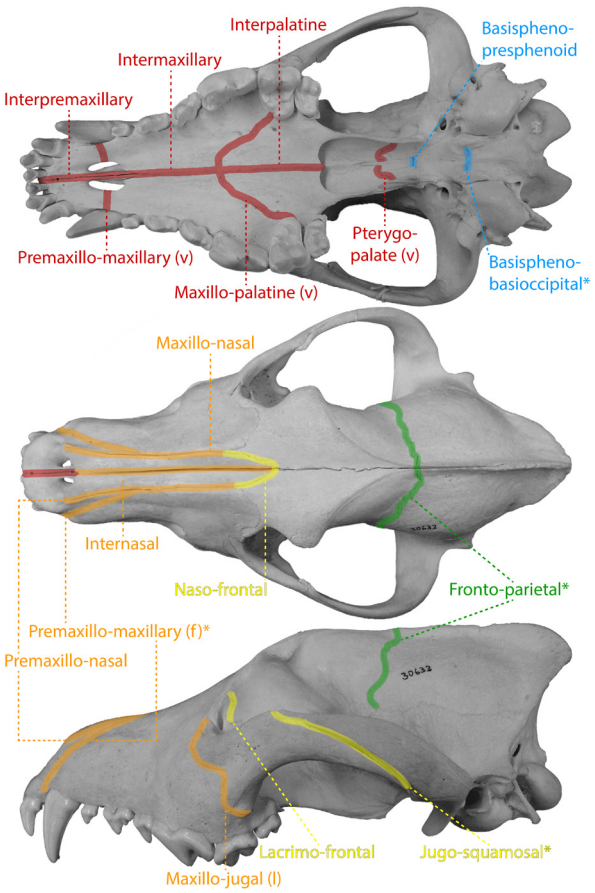

Figure 3. Cranial sutures and synchondroses coded in this study. Colours are according to functional groups by Krogman (1930): blue, basal group (cartilaginous cranial base synchondroses); red, palatal group (sutures of the rostrum in ventral aspect); orange, facial group (sutures of the rostrum, periocular area, and cheeks in dorsal or lateral aspects); yellow, cranio-facial group (sutures which connect the face to the neurocranium). Green depicts the fronto-parietal (coronal) suture. Asterisks indicate the sutures and the synchondrosis which have been found to close prematurely in humans, mice, and rabbits with a bulldog-like skull anatomy. Some sutures are denoted to be visible from frontal (f), lateral (l), and ventral (v) aspects. The exoccipito-supraoccipital, and exoccipito-basioccipital sutures are not shown here.

As a proxy for brachycephaly in this study we use the prebasial angle. All domestic dog breeds which exhibit airorhynchy are at the same time brachycephalic and represent the typical bulldog-type skull morphology, whereas not all brachycephalic breeds are at the same time airorhynch (Table 1, 2). Latter, e.g., Chihuahua, exhibit short rostra relative to the width of the skull but otherwise do not feature bulldog-type skull morphology (Fig. 2). Records of breed mean prebasial angles (angle between the hard palate and the cranial base of the skull) for the domestic dog breeds and the wolf were obtained from Nussbaumer (1982). The prebasial angle of the Chihuahua could not be obtained from the literature. It was measured in this study as described in Nussbaumer (1982) and Baxter and Nussbaumer (2009) on 19 adult Chihuahuas (complete set of fully erupted teeth and/or > 1 years old, an age at about which domestic dogs are normally fully grown; Hawthorne et al., 2004) (Table 3).

Analyses

Two sets of analyses were performed. In the first analysis, all investigated sutures were analysed together. The closure states (0, 1, or 2) for all investigated sutures and synchondroses were summed up per specimen and divided by the total number of investigated sutures (here 19). The resulting closure score is higher in old specimens with many closing and closed sutures and synchondroses and lower in young specimens with many open sutures and synchondroses. The closure scores of the airorhynch groups (boxer, Dogue de Bordeaux, French bulldog) were subsequently compared to the closure scores of the klinorhynch

Table 4. Number (n), mean age, mean prebasial angle according to Nussbaumer (1982) and this study, and associated standard deviations (sd) of specimens in this study.

Group	n	Mean age (years)	sd (mean age)	Prebasial angle (°)	sd (prebasial angle)
Barsoi	9	5.8	2.9	158.270	4.395
Bernese mountain dog	15	5.0	2.6	173.330	2.940
Boxer	13	6.7	2.8	180.900	3.872
Chihuahua	14	6.4	3.3	168.421	4.934
Chow Chow	8	6.3	2.6	175.800	4.127
Dachshund	13	6.0	2.9	165.420	4.500
Dogue de Bordeaux	7	6.1	3.2	182.250	4.628
French bulldog	14	6.5	2.7	183.050	3.199
Great Dane	12	5.6	3.1	173.470	2.183
Greyhound	14	6.0	3.0	164.410	2.272
Newfoundland	15	5.6	3.1	172.880	3.421
St. Bernard	13	5.5	3.0	177.750	4.496
Wolf	30	4.7	2.1	170.300	3.459

groups (all others). Further, all domestic dogs were compared to the wolf. For these comparisons, non-parametric Wilcoxon tests were computed.

In the second analysis, the closure states (0, 1, or 2) were summed up per suture or synchondrosis in every group. The sums were subsequently divided by the number of specimens per group. Again, the resulting closure score is higher in sutures which are closing or closed in many specimens of a group and lower in sutures which are open in many specimens of a group. Non-parametric Kendall's tau coefficients were used to investigate the correlation between the closure scores and the prebasial angle. The prebasial angles were used as the x-variables and the closure scores as the y-variable. All analyses were computed using R version 2.15.1, RStudio version 0.98.501, and Microsoft Excel 2010.

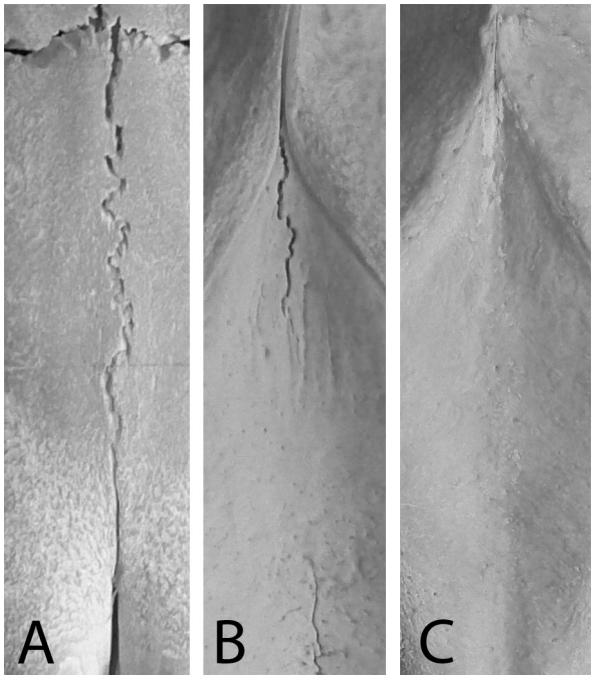

Figure 4. Illustration of a suture displaying varying levels of closure. A, open, state 0; B, closing, state 1; C, closed, state 3.

Results

Comparing the closure scores of the airorhynch (mean = 1.13, standard deviation (sd) = 0.47) versus the klinorhynch (mean = 0.8, sd = 0.37) domestic dog breeds, we found a significant difference between the two ($W = 2710$, $p = 0.0004$), showing that adults of bulldog-type brachycephalic breeds have more closing and closed sutures and synchondroses than klinorhynch breeds (Fig. 5). Moreover, we found that all domestic dogs (mean = 0.88, sd = 0.42) have significantly more closing and closed sutures ($W = 3960.5$, $p < 0.0001$) than the wolf (mean = 0.38, sd = 0.18) (Fig. 5), even when the airorhynch domestic dogs

were excluded from the comparison and ($W = 3002.5$, $p < 0.0001$).

Further, we found significant positive correlations of the prebasial angle with the closure scores of the fronto-parietal suture ($\tau = 0.51$, $p = 0.015$), premaxillo-maxillary (v) suture ($\tau = 0.61$, $p = 0.004$), premaxillo-nasal suture ($\tau = 0.68$, $p = 0.001$), maxillo-nasal suture ($\tau = 0.70$, $p = 0.001$), and basispheno-presphenoid synchondrosis ($\tau = 0.56$, $p = 0.007$).

Discussion

Characteristics of the skull and the teeth in some bulldog-like breeds of dogs and humans with achondroplasia or other syndromes related to premature closure of cranial sutures and synchondroses are strikingly similar (Fig. 1). However, patterns of suture and synchondroses closure in domestic dog breeds exhibiting bulldog-type skull conformations remain understudied. The aim of this study was therefore to investigate the breed specific differences of cranial suture and synchondroses obliteration and its relationships to brachycephaly. For this purpose the cranial sutures and synchondroses in 12 modern domestic dog breeds and the wolf were coded (Fig. 2, 3). The relative amount of closing and closed sutures in adult individuals was analysed in the context of variation of the prebasial angle, which is a proxy for brachycephalic bulldog-type skull morphology.

Our result that the closure score of the fronto-parietal suture is significantly positively correlated with the prebasial angle is consistent with experimental studies on rabbits in which it has been shown that premature closure of the fronto-parietal suture leads to an increased cranial base angle (Babler and Persing, 1982; Babler, 1988). Further, we could determine a significant positive correlation of the prebasial angle and the amount of closing and closed basispheno-presphenoid synchondrosis in domestic dog breeds. This result is consistent with findings by Schmidt et al., (2013). The probability of finding a closed spheno-occipital synchondrosis at a given age was significantly higher in brachycephalic

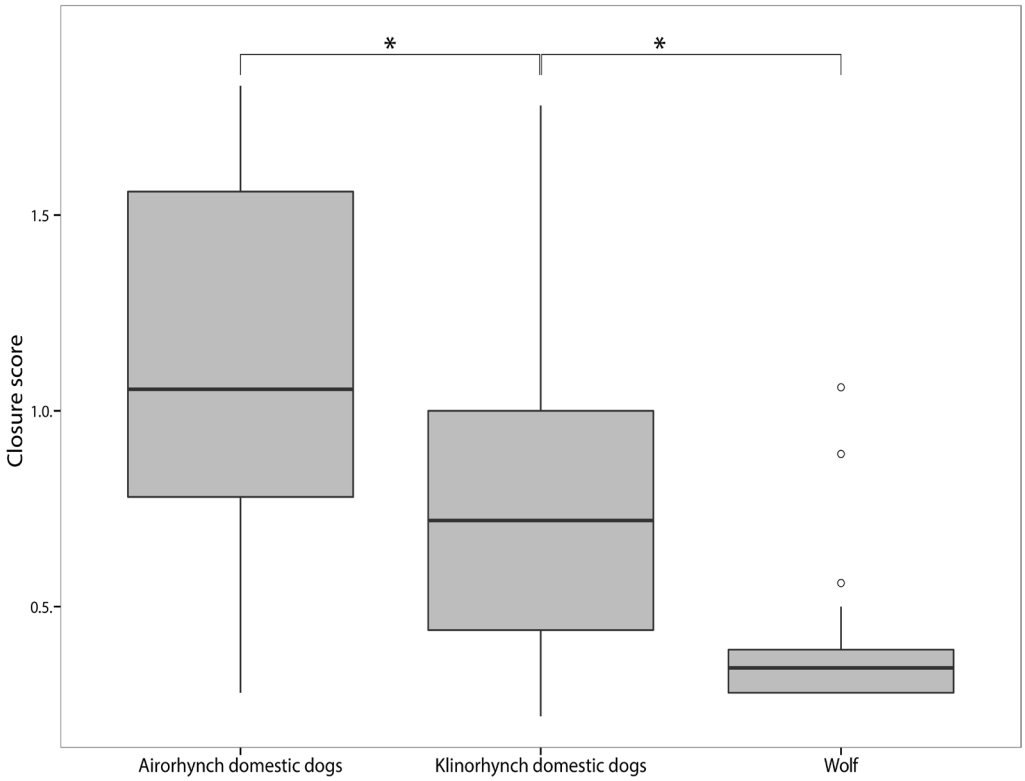

Figure 5. Relative amount (closure score) of closing and closed sutures and synchondroses in klinorhynch and airorhynch domestic dogs and the wolf. Significant differences are indicated with asterisk.

breeds compared to mesocephalic breeds in their study. Further, our results are in accordance with (1) the observed premature fusion of the fronto-parietal suture in humans with Apert and Pfeiffer syndromes that are associated with midfacial hypoplasia and (2) the observed correlation of defective endochondral ossification of the cranial synchondroses and shortening of the facial bones in humans (Cohen and MacLean, 2000; Nie, 2005; Rice, 2008a).

Our finding that breeds with a brachycephalic bulldog-type skull morphology feature significantly more closing and closed sutures and synchondroses than other breeds (Fig. 5) and that closure scores of single sutures of the rostrum are significantly positively correlated with the prebasial angle, underpin the general association of peculiar patterns of suture and synchondrosis closure in association with the brachycephalic phenotype. However, the causality of airorhynchy and patterns of suture closure in domestic dogs is not clear. It remains uncertain whether airorhynchy, or mechanisms leading to airorhynchy, also promote the great amount of obliteration of sutures of the rostrum and synchondroses, or whether airorhynchy is caused by the great amount of obliteration in facial and palatal sutures and synchondroses. Here, biomechanical aspects should be considered. Sutures are importance sites of flexibility which transmit loads during muscle activity or traumatic impacts (Herring, 2008). It has been found that patent sutures lead to a wide distribution of strain around the skull during biting in reptiles (Moazen et al., 2009; Curtis et al., 2013) and to great deformations in Macaque skulls, although the significance of the effect of open sutures is not clear (Wang et al., 2010). Studies on mice have shown that reduced masticatory function leads to early internasal suture closure (Engström et al., 1986). Moreover, the report of highly different degrees of suture closure in the skulls of two large pinnipeds *Mirounga leonina* and *Odobenus rosmarus*, which exhibit different feeding strategies, suggest that masticatory function may influence suture closure (Goswami et al., 2013). Differences in feeding strategy and lifestyle might thus have an influence on the amount of suture closure, also in domestic dogs and the wolf, and could explain our finding of generally few closed sutures in the wolf compared to domestic dogs and the difference of closure scores in klinorhynch vs. airorhynch domestic dog breeds (Fig. 5). The wolf is an active predator of mainly large mammals and it usually consumes the entire carcass including the bones (Peters, 1993; Nowak, 1999). In modern European domestic dogs, as all specimens in our sample, hunting is not the primary source of food and it can be assumed that the nutrition of most of these specimens did not regularly include bones. Thus, stress regimes in the skulls of domestic dogs and wolves are likely different, probably leading to a greater amount of suture obliteration in domestic dogs due to reduced stress acting on their skulls. Brachycephalic domestic dogs usually exhibit mandibular protrusion and dental malocclusion and are reported to have often difficulties with eating (Fasanella et al., 2010). Even further reduced mechanical stresses on the skulls of brachycephalic breeds might thus lead to an even greater amount of suture closure in these breeds.

In contrast to other studies on humans, mice, and rabbits (see Introduction), we did not find a greater amount of closure in the basispheno-basioccipital synchondrosis, facial portion of the premaxillo-maxillary suture, and jugo-squamosal suture. Certainly, the observed patterns of suture and synchondrosis closure are linked to species specific modes of growth and direct comparisons of these patterns among the species remain problematic. For example, it should be considered that the brachycephalic and airorhynch phenotypes are not only caused by premature closure of sutures and synchondroses (Cohen and MacLean, 2000). Midface hypoplasia can be one symptom of cleidocranial dysplasia, a syndrome in humans which is, amongst other

symptoms, characterised by unossified sutures and fontanelles. Cleidocranial dysplasia is caused by a heterozygous loss-of-function mutation in the Runt-related transcription factor 2 (RUNX2), an important regulator of osteoblast differentiation which is not associated with prematurely closing sutures (Mundlos, 1999; Cohen and MacLean, 2000; Schroeder et al., 2005; O'Neill, 2013a, 2013b). The glutamine-alanine tandem-repeat ratio in the domestic dog RUNX2 orthologue was found to be positively correlated with midface length and degree of klinorhynchy in domestic dogs (Fondon and Garner, 2004). Similar results were reported for other carnivoran taxa (Sears et al., 2007), but not across other placental clades (Pointer et al., 2012).

To conclude, we detected a greater amount of suture and synchondroses closure in brachycephalic bulldog-type than in other domestic dog breeds, especially regarding the fronto-parietal suture and the basispheno-presphenoid synchondrosis, a finding which is consistent with patterns found in humans that exhibit syndromes causing midfacial hypoplasia. An overall great amount of suture closure of some facial sutures in increasingly airorhynch breeds, on the other hand, point to biomechanical influence on patterns of suture closure in domestic dogs. Thus, brachycephaly is likely the closely and directly linked to peculiar patterns of suture and synchondrosis closure in domestic dogs.

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Supplementary material

Description of the materials used and methods applied by Nussbaumer (1982) to obtain prebasial angles of domestic dog skulls

In this study on the variability of the prebasial angle in domestic dogs, Nussbaumer (1982) compiled a comprehensive sample including 2 wild canid species (wolf and golden jackal) and 39 domestic dog breeds representing a great variety of body sizes and forms. In sum, 499 specimens, with on average 12.2 (2 to 32) specimens per breed were measured, most of them are housed in NMBE. Only adult specimens were used, i.e., specimens with fully erupted permanent teeth. The sex was not further specified, but the author found no sex-based dimorphism in the prebasial angle measurements in the wolf sample and thus did not split the domestic dog sample according to sex, even more because sexual dimorphism in general is reduced in domesticated canids.

Nussbaumer (1982) and Baxter&Nussbaumer (2009) provide detailed descriptions of the measurement of prebasial angles. Two anatomical planes on the ventral side of the skull have to be examined, the 'basisphenoid-basioccipital-plane' and the 'hard-palate-plane'. The 'basisphenoid-basioccipital-plane' is defined as the distance between the rostral border of the ventral rim of the foramen magnum to the medium point of the virtual line between the intersection points of the presphenoid, pterygoid, and palatine on the left and the right side of the skull. The 'hard-palate-plane' is defined as the distance between the medium point of the virtual line between the rostral points of the two recesses at the left and right side of the caudal nasal spine of the palatine to the medium point of the virtual line between the caudal rims of the two palatine fissures. A contour gauge was laid through the above described anatomical landmarks on the ventral surface of the skull and the resulting profile was copied to paper. The two above described planes were measured with a calliper and directly transferred to the profile on the paper. The anatomical landmarks of the two planes could then be graphically connected to each other with straight lines. Subsequently, the two planes were translated to make them touching each other and the angle between the two planes was measured with an angle gauge.

The table below lists the number of used specimens and measured prebasial angles as determined by Nussbaumer (1982) in the breeds which were examined in our study and the wolf.

Group	n	Prebasial angle (°)	sd (prebasial angle)
Barsoi	15	158.270	4.395
Bernese mountain dog	30	173.330	2.940
Boxer	20	180.900	3.872
Chow Chow	15	175.800	4.127
Dachshund	26	165.420	4.500
Dogue de Bordeaux	4	182.250	4.628
French bulldog	20	183.050	3.199
Great Dane	15	173.470	2.183
Greyhound	17	164.410	2.272
Newfoundland	30	172.880	3.421
St. Bernard	20	177.750	4.496
Wolf	32	170.300	3.459

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CHAPTER 4

Comparison of growth trajectories among ancient and modern breeds rejects a paedomorphic pattern of change associated with dog domestication

This work was done during the third year of my PhD. I collected the data, conducted the analyses, and wrote the manuscript. Both authors then participated in drafting the final manuscript.

This article is ready for submission, but we plan to gather more data.

Comparison of growth trajectories among ancient and modern breeds rejects a paedomorphic pattern of change associated with dog domestication

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There is a controversial hypothesis of an association of retardation of growth rate, late onset, or early offset of growth (heterochrony: paedomorphosis) with the generation of the typical skull shape in domestic dogs, with the latter being 'paedomorphic wolves'. Here we present the first analysis of ontogenetic series of dogs from different historical periods of domestication (modern, premodern, prehistoric), serving to provide a comprehensive test of the paedomorphosis hypothesis. The mandible is also investigated, to examine the complete picture of morphological disparity of skull shape. We used three-dimensional landmark-based coordinate data from 106 crania and 93 mandibulae of ontogenetic series of domestic dogs and wolves and found that adult cranial and mandibular shape in some, mainly premodern domestic dogs, is similar to wolves, but does not intersect with the ancestral ontogenetic trajectory. In cases where ontogenetic trajectories overlap (German shepherd), these trajectories are similar to each other, indicating similar patterns of growth. Therefore, we did not find evidence for heterochrony in the investigated groups and conclude that domestic dogs are not paedomorphic wolves.

Keywords: Domestication, wolf, heterochrony, geometric morphometrics

Introduction

Domesticated mammals are characterised by a set of morphological and behavioural peculiarities which cannot be regularly observed in their wild ancestors [1-3]. One mechanism generating disparity is neoteny, a retardation of growth rate and retention of juvenile characteristics into adulthood [4, 5], a process which has been suggested to be responsible for anatomical and behavioural disparity between the domestic dog and its ancestor, the wolf (*Canis lupus*) [6-13]. Although the similarity between adult dogs and juvenile wolves (short face and broad skull) has been suggested by many authors, the hypothesis has also been challenged [14, 15, 9, 16]. A morphometric study [16] found that the skull shape in domestic dogs is neomorphic compared to wolves and not a result of heterochronic processes. Neither the small angle between the nasal/maxillary and the frontal bones (pronounced 'stop') of many breeds nor the extreme dorsal (airorhynchy) or ventral (klinorhynchy) orientation of the rostrum in relation to the base of the skull occur in any ontogenetic stage of the wolf [16]. Challenges to the paedomorphosis hypothesis, although not quantified by statistical analyses, had already been presented [14, 15] and it was found that the skull shape of domestic dogs of different breeds is already very distinct at birth and continues to grow differently during postnatal ontogeny.

The study by Drake [16] constitutes a significant contribution to this debate by applying a geometric morphometrics approach, making possible the analysis of the pure shape [17] instead of the traditionally used linear distances [18]. However, the previous studies can be expanded significantly to address the issue at hand, including consideration of several fundamental aspects, such as the pooling by breed and not by skull type (which leads

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to an oversimplification), the examination of ontogenetic series of domestic dogs (because the comparison of ontogenetic trajectories is crucial for the investigation of heterochronic patterns [18]), and the examination of the mandibles (because mandible and skull develop as separate and independent characters [19]). The aim of this study is therefore to investigate ontogenetic skull shape variation in domestic dogs through the analysis of cranial shapes pooled by dog type/breed, incorporating domestic dogs from different historical periods (modern, premodern, prehistoric), investigating ontogenetic trajectories of domestic dogs, and investigating shape changes in the mandible.

Materials and Methods

The three-dimensional coordinates of 38 cranial and 10 mandibular landmarks (Fig. S1, Table S1) were captured with a MicroScribe MX 3D digitiser (Solution Technologies, Inc.) on 106 crania (dorsal and ventral aspects) and 93 mandibulae of ontogenetic series of domestic dogs and wolves (Table S2). The choice of landmarks was based on their recognisability in all age stages (juvenile to late adult).

Dog breed/type selection was deliberately biased to choose those representing different historical periods. 'Modern' breeds are recognised by kennel clubs and have been subject to exhaustive breeding regimes with strict aesthetic requirements and closed bloodlines during the last approximately 150 years [20]. They therefore presumably exhibit a derived skull and mandibular conformation. 'Premodern' domestic dogs are here defined as dogs from a time before the establishment of kennel clubs, i.e., before about the mid-19th century. These dogs have been bred and classified according to their function and there was usually no strict selection for aesthetics traits, e.g., ratter dogs and pointing dogs were used for rat hunting and finding prey during a hunt, respectively. The premodern group further contains 'basal' domestic dogs. These are groups of dogs, e.g., Australian dingo and New Guinea singing dog, that are geographically and/or culturally isolated from the modern domestic dog breeds and thus occupy well-supported basal positions on phylogenetic trees [20]. We assume that premodern dogs have less derived skull and mandibular shapes than the modern breeds. 'Prehistoric' domestic dogs represent the oldest group of dogs from the Stone, Bronze, and Iron ages. Their skull and mandibular shape reflect the most ancestral, condition relatively early in the domestication process because directed and conscious artificial selection did probably not yet play a major role in the generation of skull shapes. Instead, natural selection might have been more important. It has to be kept in mind that natural selection, not artificial selection, is probably also the main generator of skull and mandibular shape in the premodern Australian dingo and the New Guinea singing dog. Heterochronic patterns, if present, were most probably already established in these prehistoric domestic dogs, since already the oldest archaeological remains show skull conformations resembling recent domestic dogs [8, 21, 22]. Due to the presumably different selection regimes during these historical periods, it is crucial to investigate heterochronic patterns separately in modern, premodern, and prehistoric domestic dogs. The domestic dog sample used here consists of modern breeds (French bulldog, German shepherd, poodles), premodern dogs (pointing dogs, ratter dogs, Australian dingo, Afghan hound, Akita, New Guinea singing dog [23, 24, 20]), and prehistoric dogs. The latter group includes one skull and mandible of an Iron Age dog and several skulls and one mandible of Neolithic turbary dogs found around the Bielersee, Switzerland. Originally, the Iron Age dog was described as *Canis familiaris intermedius* and the Neolithic skulls as *C. f. palustris* according to Studer [25]. Since these designations are obsolete we refer to these skulls as 'intermedius' or 'palustris' type, respectively. The ventral and the dorsal aspects of the crania were combined

into one set of coordinates for the entire skull through digitising three landmarks from both aspects. The left and the right side were digitised in the crania; only the right ramus was digitised in the mandibulae. Every cranium and mandible was digitised twice to account for random errors in individual measurements during digitisation. Examined individuals were classified as juvenile or adult, depending on if the complete set of permanent teeth is fully erupted (adult) or not (juvenile).Specimens are housed in Albert-Heim-Stiftung, Schweiz (NMBE); (former) Institut für Haustierkunde (now Zoologisches Institut, Populationsgenetik), Christian-Albrechts-Universität zu Kiel, Germany (I.f.H.); Naturhistorisches Museum Bern, Schweiz (NMBE); Paläontologisches Institut und Museum der Universität Zürich, Schweiz (PIM); Zoologisches Museum der Universität Zürich, Schweiz (ZMUZH).

All analyses were conducted using Microsoft Excel 2010, R version 3.1.3 [26], RStudio version 0.98.1103 [27], and MorphoJ version 1.05f [28]. To determine the amount of measurement error between the two replicates in every specimen, Procrustes ANOVA was calculated [29]. The amount of shape variation due to digitising error in the skull and the mandible is 13 - 100 and 46 – 9653 times, respectively, smaller than any biological variation (among individuals and/or between the two sides of the skull) and is thus negligible. The mean of both replicates was calculated for every landmark in all specimens. The complete dataset is available as part of the supplementary data (Table S3).

Few specimens were not complete, i.e., not all landmark positions were measurable. In these specimens the missing landmarks were estimated using the "estimate.missing" function in the geomorph-package version 2.1.4 [30] in R. This function interpolates the coordinates of a landmark of a reference specimen (obtained from a set of specimens for which all landmarks are present) on the specimen with missing landmarks using the thin-plate spline.

Two general sets of analyses were conducted: ontogenetic series of wolves were compared with (1) adult domestic dogs and (2) ontogenetic series of domestic dogs. For the latter, the 'intermedius' and 'palustris' dogs as well as the miniature and standard poodles had to be excluded because juveniles were not available. Within both sets of analyses, four subsets of the data were analysed: (a) all landmarks of the cranium, (b) landmarks of the neurocranium, (c) landmarks of the face, and (d) landmarks of the mandible. The subsets that distinguish between neurocranium and face (Fig. S1) were chosen because they have been found to comprise developmental modules in domestic dogs [31] and it is to be expected that heterochrony does not affect the skull as a whole but only certain parts of it [16]. Further, every dataset was analysed using allometric and the non-allometric shape component (see below). In sum, 16 sets of analyses were conducted (Table 1 & 2).

The following analyses were conducted for all of above described datasets. Generalized Procrustes superimpositions were computed to extract geometric shape of the investigated specimens simultaneously. Subsequently, a covariance matrix of the symmetric component of averaged Procrustes shape coordinates (average of the left and right sides of the skull) was generated. Principal component analyses (PCA) based on these covariance matrices were performed to explore patterns of shape variation. The effect of allometry on shape was determined by a multivariate, pooled (within group) linear regression of the Procrustes shape coordinates against log centroid size (CS) with 10'000 permutations [32]. The residuals of these regressions were also used for PCA to investigate the non-allometric shape component of our data. To determine similarity of angles between ontogenetic trajectories, regressions of shape on log CS were performed within groups with 10'000 permutations in a single multidimensional shape space [32]. The angles between the centered origins of the regressions were then compared.

Canonical variate analyses were performed to quantify Procrustes distances between pre-defined groups (modern, premodern, prehistoric) with associated significance values using 10'000 permutations [33]. To affirm that the neurocranial and facial modules can be distinguished in our sample, we calculated the RV coefficient using the regression residuals of all adult specimens [34].

Results & Discussion

In our ontogenetic series of wolves and adult domestic dogs, allometry explains 19.3% of the total variation ($p<.0001$). We found an RV coefficient of 0.73 between the facial and neurocranial landmarks, which is lower than for most other subdivisions of landmarks ($p = 0.01$). This result is in agreement with Drake and Klingenberg [31], and shows that, although the integration between face and neurocranium is strong, there is some degree of modularity which legitimates their separate examination.

We detected similarity of skull shape in some groups of adult domestic dogs compared to the wolf ontogenetic series (Table 1). This finding is in contrast to Drake [16] who found that the differences of cranial shape between wolf ontogenetic series and adult domestic dogs are significant.. These similarities were discovered considering the whole cranium as well as neurocranium and face separately and considering allometric and non-allometric data (Table 1). Notably, the groups that are similar to the wolf ontogenetic series in all settings are not members of modern breeds but of prehistoric 'intermedius' type dog and the premodern Australian dingo and pointing dog (Table 1). When only the neurocranium is included, several other domestic dog groups are similar to the wolf (Table 1). Comparisons of mandibular shape of adult domestic dogs and ontogenetic trajectories of wolves revealed similarities of wolf and German shepherd (Table 1).

PC1 is associated with a elongation, narrowing, and flattening of the skull as well as with an increased ventral rotation of the rostrum relative to the cranial base (Fig. 1a). These shape changes are strongly influenced by the typical bulldog-like (brachycephalic – short face and wide skull – and airorhynch) skull shape of the French bulldog. PC2 is related to an elongation and narrowing of the rostrum, a small angle between the nasal/maxillary and the frontal bones ('stop'), an increased ventral rotation of the rostrum relative to the cranial base (klinorhynchy), and a narrowing of the zygomatic arches, most probably influenced by the generally more klinorhynch condition of the juvenile compared to adult wolves [15] (Fig. 1a). The French bulldog, the only brachycephalic breed, is widely separated from all other specimens, juvenile and adult, which are mesocephalic (typical canine skull proportions) (Fig. 1). Similar results were found if the allometric component was removed from the data (Fig. S2). The non-allometric comparisons make evident that a great portion of shape variation among the groups is influenced by differences in size, as the shape space occupation decreases when the allometric component is removed (Fig. S2). Concerning the mandible, PC1 is associated with a ventral bending and mediolateral broadening of the dentary, most probably influenced by the usually bended mandibulae of the French bulldog, as well as with a heightening of the coronoid process and a widening of the condyloid process (Fig. 1b). PC2 is associated with an anterior bending of the coronoid process and a dorsoventral widening of the dentary. The mandibular shape space is smaller than cranial shape space, implying a lower disparity of mandibular shape despite the simultaneous great disparity in skull shape (Fig. 1, S2, S3).

Despite their similarity when comparing Procrustes distances, none of the investigated breeds shares its entire morphospace with the ontogenetic trajectory of the wolf (PC1 – 3, allometric and non-allometric shape space of adult dogs and ontogenetic series of wolves, Fig. 1, Fig. S2), a result which is in congru-

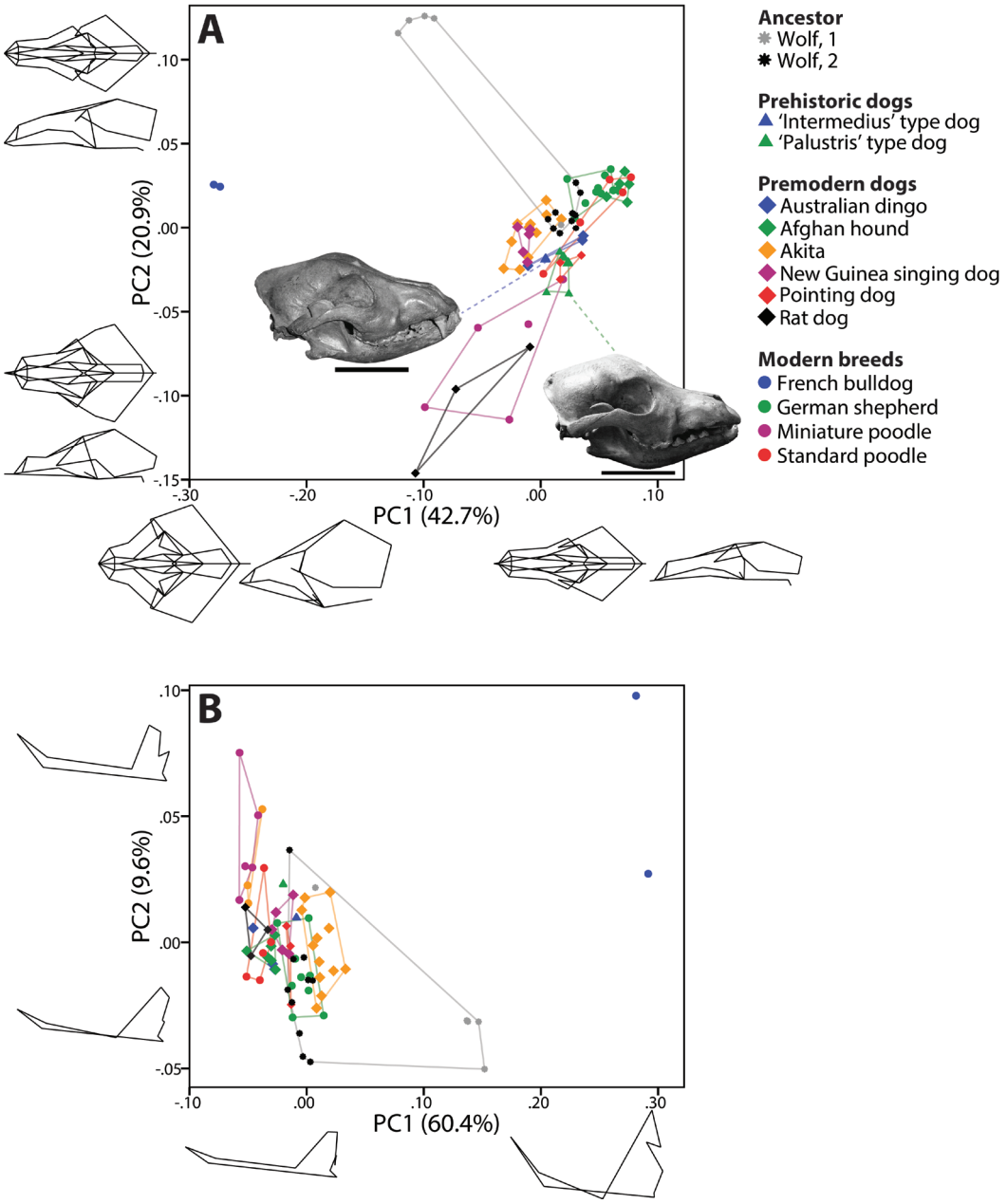

Figure 1. Principal component analysis of cranial shape (A) and mandibular shape (B) comparing the ontogenetic series of the wolf to different groups of adult domestic dogs. Photographs depict skulls of the 'intermedius' type Iron Age dog and a 'palustris' type Neolithic dog. Schematic outlines of crania and mandibluae represent extreme shape forms on PC1 and PC2.

ence with the results by Drake [16]. The prehistoric dogs do not occupy positions in shape space more similar to the wolf than recent groups, e.g., German shepherd, standard poodle, Akita (Fig. 1, Fig. S2). More importantly, they do not cluster with juvenile wolves, which would point towards a juvenilisation of the skull shape during early domestication. Similar patterns as in the skull can be detected regarding the mandibular shape space (PC1 – 3, allometric and non-allometric shape space), although here, overlapping is more extensive for certain groups (Akita, German shepherd, pointing dog, and 'intermedius' type dog; Fig. 1, Fig. S3). However, also here all domestic dogs cluster with adult wolves, rather than with juveniles.

Table 1. Cranial and mandibular shape differences between ontogenetic series of wolves and adult domestic dogs. Non-significant p-values are indicated in grey. Proc. D., Procrustes distances; p, significance values.

	Whole cranium		Neuro-cranium		Face		Mandible	
	Proc. D.	p	Proc. D.	p	Proc. D.	p	Proc. D.	p
(a) Procrustes shape variation								
Australian dingo	0.08	0.1396	0.07	0.271	0.09	0.1275	0.08	0.1246
Afghan hound	0.10	0.0044	0.08	0.0671	0.12	0.0029	0.08	0.0147
Akita	0.08	0.0002	0.08	0.0095	0.10	<.0001	0.06	0.0268
French bulldog	0.28	0.0026	0.27	0.0081	0.30	0.0057	0.27	0.0091
German shepherd	0.07	0.0153	0.07	0.0406	0.09	0.017	0.05	0.0588
‚Intermedius' type dog	0.09	0.3789	0.11	0.4623	0.10	0.4086	0.07	0.6923
Medium poodle	na	na	na	na	na	na	0.10	0.0334
Miniature poodle	0.13	0.0011	0.15	0.0014	0.15	0.0014	0.11	0.0027
New Guinea singing dog	0.07	0.041	0.08	0.0433	0.09	0.0391	0.07	0.0388
‚Palustris' type dog	0.09	0.0346	0.09	0.0406	0.10	0.0384	0.08	0.4383
Pointing dog	0.09	0.1223	0.11	0.0809	0.10	0.1139	0.07	0.1459
Ratter dog	0.16	0.0049	0.13	0.0093		0.0044	0.10	0.0517
Standard poodle	0.08	0.0394	0.09	0.0439	0.10	0.0336	0.08	0.0347
(b) Nonnallometric shape variation								
Australian dingo	0.08	0.1301	0.08	0.164	0.09	0.1238	0.10	0.0106
Afghan hound	0.11	0.0036	0.07	0.0736	0.13	0.0026	0.09	0.0059
Akita	0.07	0.0011	0.09	0.0004	0.09	0.0008	0.06	0.0023
French bulldog	0.24	0.0013	0.27	0.0092	0.25	0.0074	0.22	0.0084
German shepherd	0.08	0.0139	0.07	0.047	0.09	0.0132	0.06	0.0213
‚Intermedius' type dog	0.09	0.3834	0.11	0.327	0.09	0.4814	0.08	0.3766
Medium poodle	na	na	na	na	na	na	0.14	0.0067
Miniature poodle	0.07	0.0616	0.05	0.4641	0.09	0.041	0.18	<.0001
New Guinea singing dog	0.07	0.0445	0.09	0.0407	0.06	0.3677	0.11	0.0031
‚Palustris' type dog	0.10	0.0347	0.09	0.0398	0.11	0.0377	0.10	0.3095
Pointing dog	0.09	0.1204	0.10	0.0864	0.10	0.1172	0.07	0.1088
Ratter dog	0.08	0.1422	0.09	0.1652	0.10	0.1191	0.18	0.0006
Standard poodle	0.10	0.0345	0.07	0.1502	0.12	0.0151	0.10	0.0063

The ontogenetic trajectories of most domestic dogs take different directions than the wolf trajectory but most of them converge (except the French bulldog) (Fig. 2). Heterochrony is a valid description if ontogenetic trajectories overlap in shape space but diverge in size-shape space (different angles) [5, 18]. Ontogenetic series reveal that only the growth trajectory of the German shepherd is congruent with the wolf trajectory in shape space when comparing PC1 vs. PC2 (Table 2, Fig. 2). If trajectories in PC1 vs. PC3 are visually compared, there is no apparent similarity of trajectories (Fig. S4). The angle of their ontogenetic trajectories in size-shape space is more similar than expected by chance ($p<.00001$), leaving no evidence for heterochrony. In contrast, the ontogenetic trajectories of the mandible in German shepherds and wolves overlap completely when comparing PC1 vs. PC2 (Fig. 2) and PC1 vs. PC3 (Fig. S5) and also Procrustes distances are similar (Table 2). The angles between the two trajectories in size-shape space are not more similar than expected by chance ($p = 0.93$). Similar growth trajectories and dissimilar angles between them render heterochrony a valid description [18]. The somewhat steeper regression slope of the German

Table 2. Cranial and mandibular shape differences between ontogenetic series of wolves and ontogenetic series of domestic dogs. Non-significant p-values are indicated in grey. Proc. D., Procrustes distances; p, significance values.

	Whole cranim		Neuro-cranium		Face		Mandible	
	Proc. D.	p	Proc. D.	p	Proc. D.	p	Proc. D.	p
(a) Procrustes shape variation								
Australian dingo	0.10	0.0069	0.07	0.2103	0.12	0.0037	0.09	0.0322
Afghan hound	0.08	0.0105	0.08	0.0207	0.10	0.0112	0.09	0.0065
Akita	0.09	<.0001	0.07	0.011	0.12	<.0001	0.06	0.0048
French bulldog	0.29	<.0001	0.28	<.0001	0.32	<.0001	0.26	<.0001
German shepherd	0.05	0.154	0.06	0.1157	0.06	0.1678	0.03	0.4083
Medium poodle	na	na	na	na	na	na	0.10	0.0186
New Guinea singing dog	0.07	0.0128	0.08	0.0135	0.09	0.0122	0.08	0.0106
Pointing dog	0.08	0.0094	0.10	0.0071	0.09	0.0113	0.07	0.01
Ratter dog	0.15	<.0001	0.14	0.0009	0.18	<.0001	0.10	0.0037
(b) Nonnallometric shape variation								
Australian dingo	0.11	0.0034	0.08	0.07	0.12	0.0046	0.09	0.0185
Afghan hound	0.11	0.0007	0.07	0.0923	0.13	0.0002	0.09	0.0065
Akita	0.08	<.0001	0.08	0.0014	0.10	<.0001	0.07	0.0001
French bulldog	0.21	<.0001	0.25	<.0001	0.23	<.0001	0.25	<.0001
German shepherd	0.07	0.0238	0.06	0.1357	0.07	0.0392	0.03	0.4379
Medium poodle	na	na	na	na	na	na	0.11	0.0174
New Guinea singing dog	0.09	0.0018	0.10	0.0012	0.09	0.0152	0.09	0.0019
Pointing dog	0.09	0.0089	0.10	0.0083	0.10	0.008	0.08	0.009
Ratter dog	0.12	0.002	0.08	0.0651	0.15	0.0017	0.12	0.0022

shepherd (Fig. S6) points to an acceleration of growth and thus peramorphosis in the German shepherd compared to the wolf [5]. However, we think that this finding is a sampling artefact since the youngest Germans shepherd is only about one month old whereas the youngest wolves are about three months old. Otherwise, similar age classes of German shepherd and wolf cluster together in the size-shape space (Fig. S6). Moreover, adult specimens of both groups share a similar position in the size-shape space, which contradicts the assessment of the mandibular shape of the German shepherd as peramorphic

To conclude, we found no evidence for heterochrony in the skulls and mandibles of different groups of domestic dogs representing different stages during the domestication process and we can confirm the findings by Drake [16] and earlier authors [14, 15] that domestic dogs are not paedomorphic wolves. However, we cannot confirm that domestic dogs exhibit a de novo rearrangement of the skull per se. Instead, some domestic dogs, especially the ones that share a similar adult cranial and mandibular shape with the wolf, e.g., German shepherd, feature similar skull and mandibular shapes and ontogenetic trajectories. Since the wolf-like appearance of the German shepherd is a product of modern breeding regimes, the resemblance of the ontogenetic trajectories is probably a secondary reestablishment of the ancestral condition. Convergences in allometric patterns of growth have been reported for other groups, as driven by ecology [35], pointing out the mixed developmental and functional component of the multivariate growth process [36].

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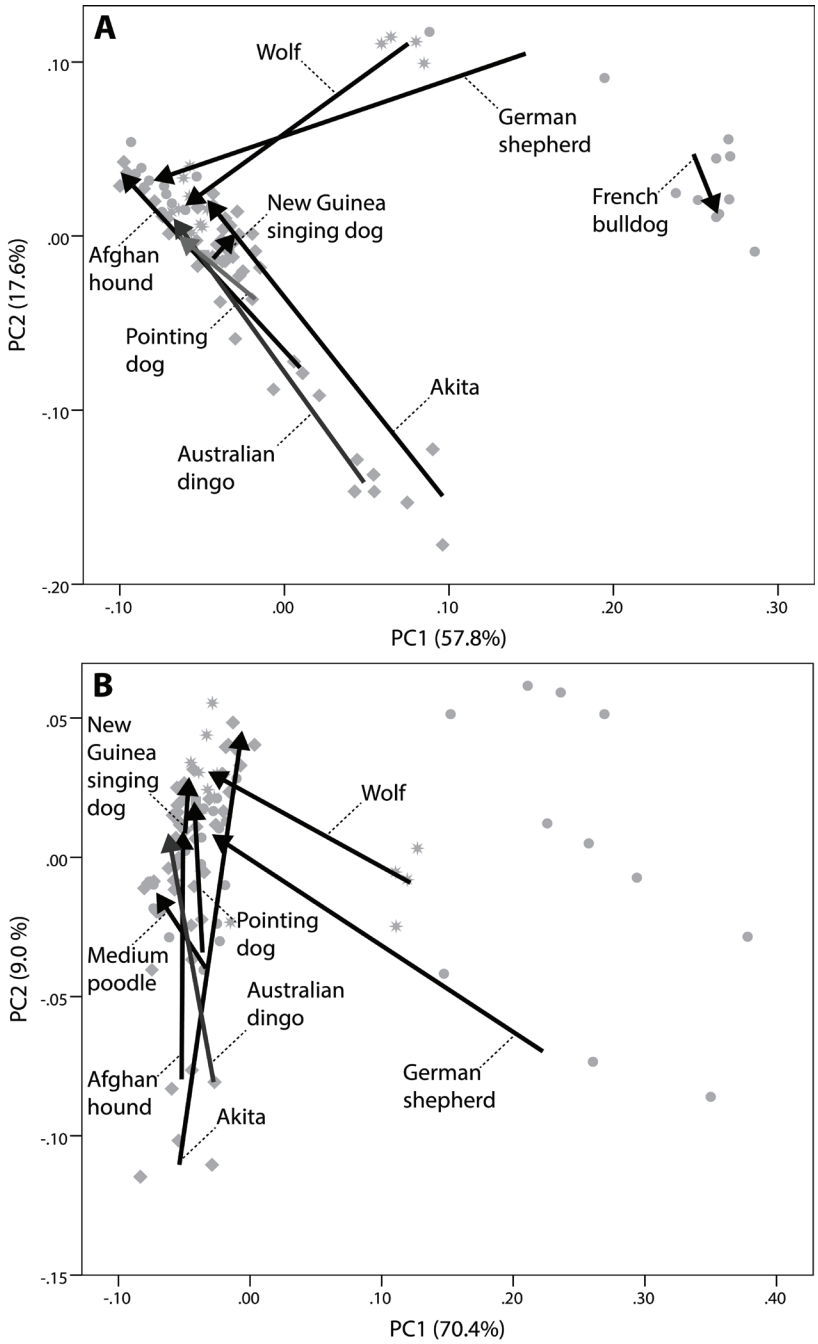

Figure 2. Principal component analysis of cranial shape (A) and mandibular shape (B) comparing the ontogenetic trajectory of the wolf to ontogenetic trajectories of different groups of domestic dogs (no arrow is assigned to groups in which ontogenetic trajectories cannot be discerned). Black arrows indicate the direction of the ontogenetic trajectories. Corresponding colour figures and legend can be found in Figure S3.

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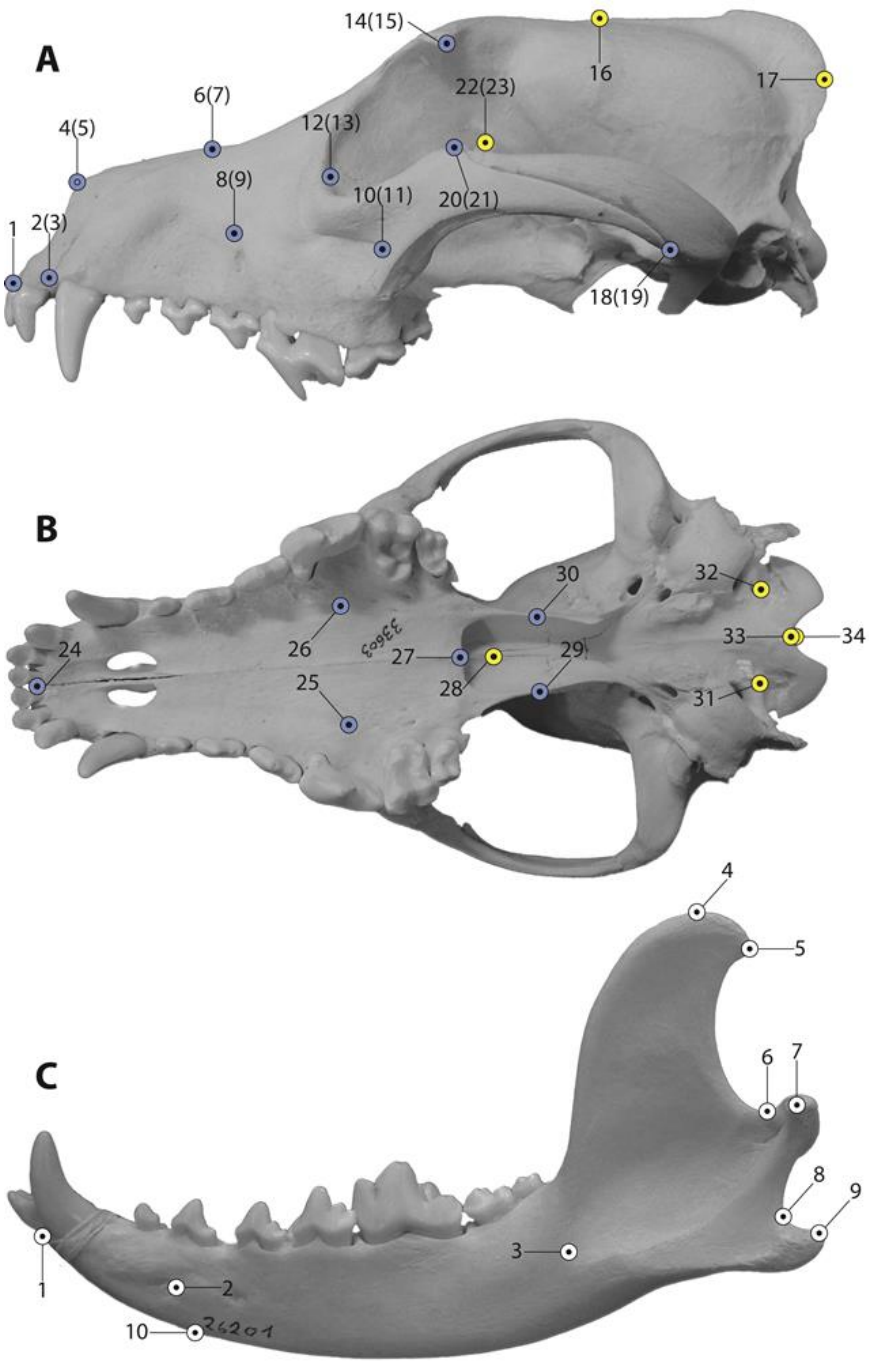

Figure S1. Landmarks used in this study displayed on a skull in lateral (A) and ventral (B) aspects and on a mandible (C). Numbers correspond to Table S1 and numbers in brackets refer to the respective contralateral landmark. Blue, landmarks of the facial module; yellow, landmarks of the neurocranial module.

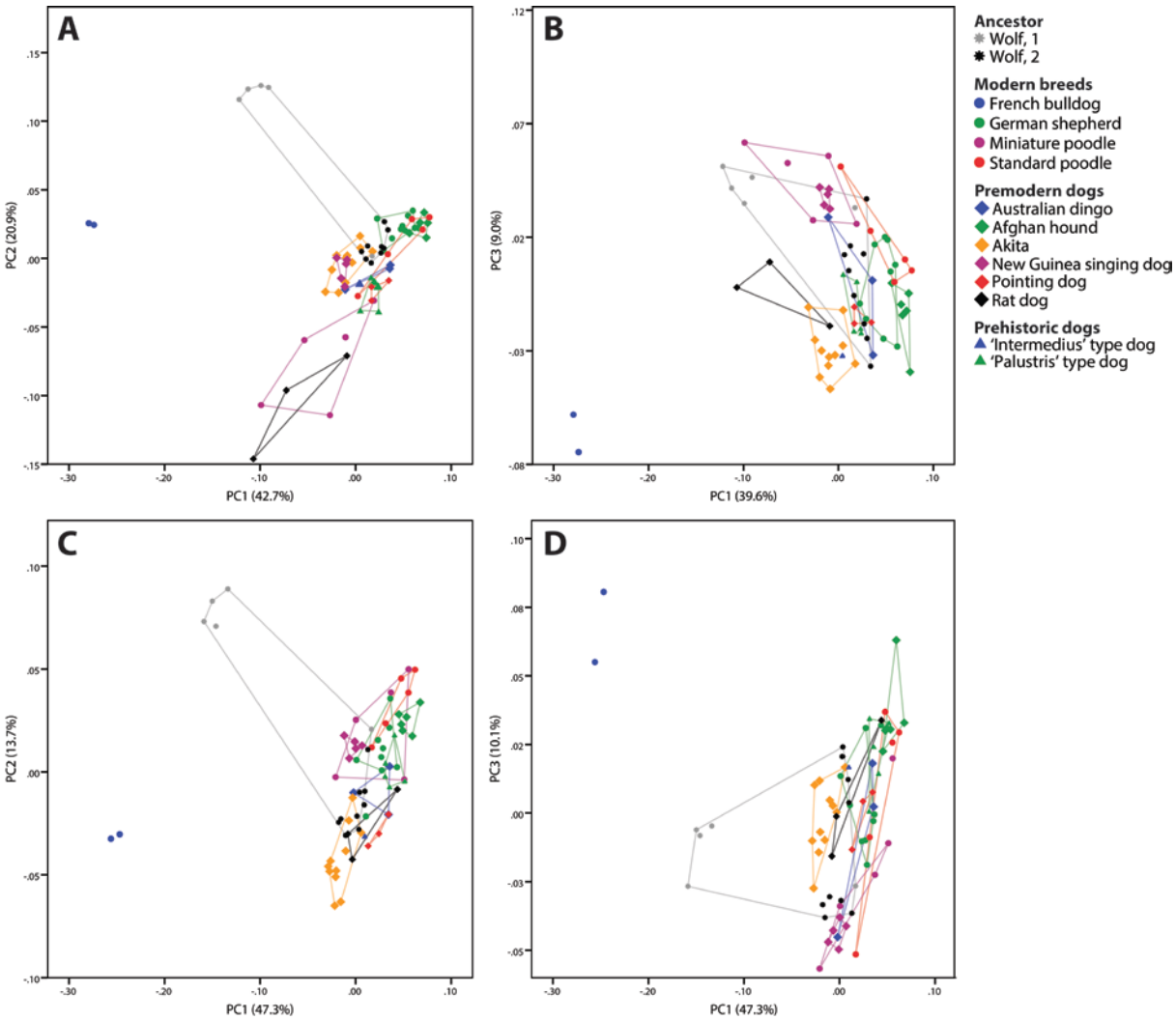

Figure S2. Principal component analysis of cranial shape comparing the ontogenetic series of the wolf to different groups of adult domestic dogs. A / B and C / D depict the allometric and non-allometric components of cranial shape variation, respectively.

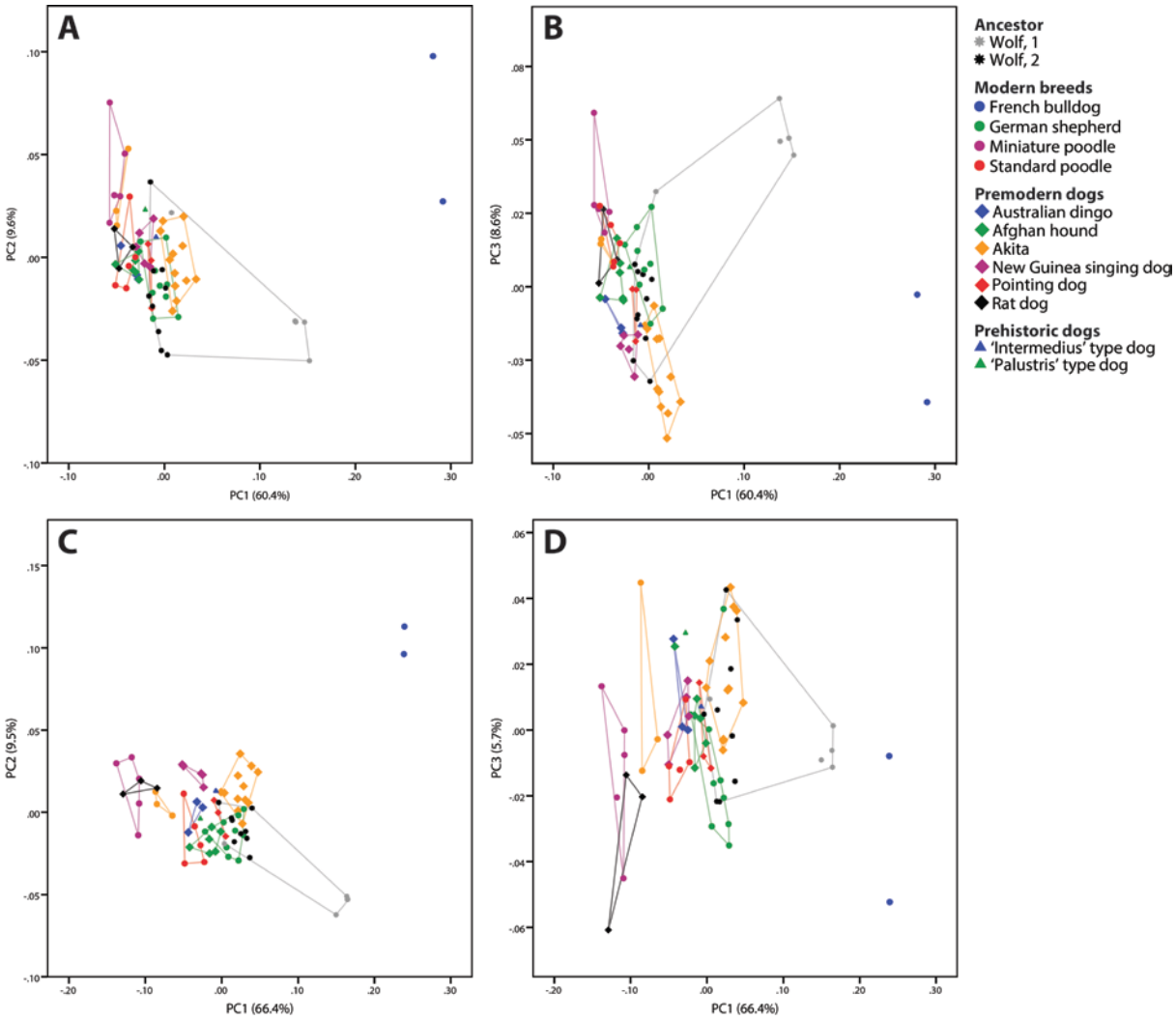

Figure S3. Principal component analysis of mandibular shape comparing the ontogenetic series of the wolf to different groups of adult domestic dogs. A / B and C / D depict the allometric and non-allometric components of mandibular shape variation, respectively.

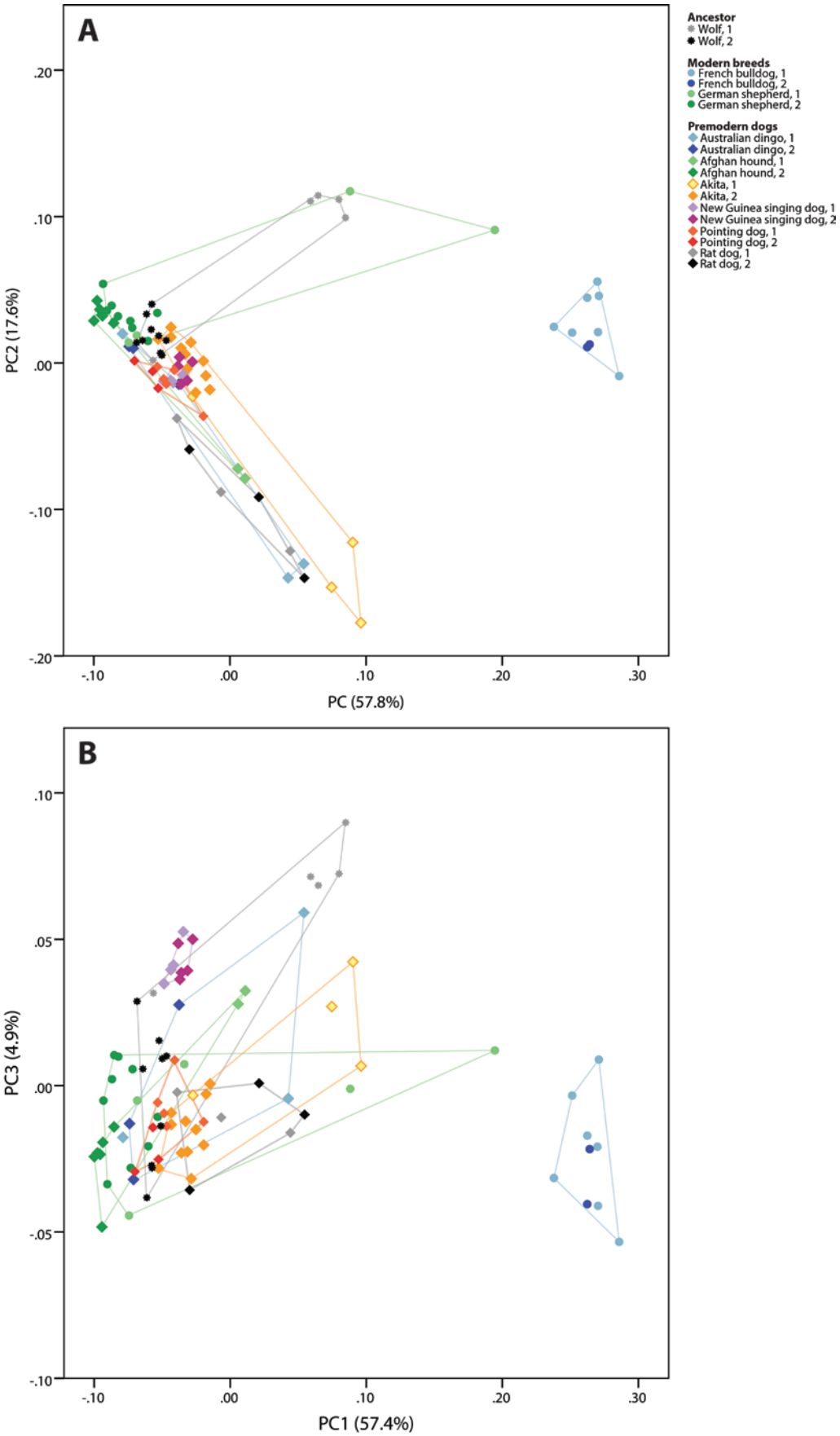

Figure S4. Principal component analysis of cranial shape comparing the ontogenetic series of the wolf to the ontogenetic series of different groups of domestic dogs.

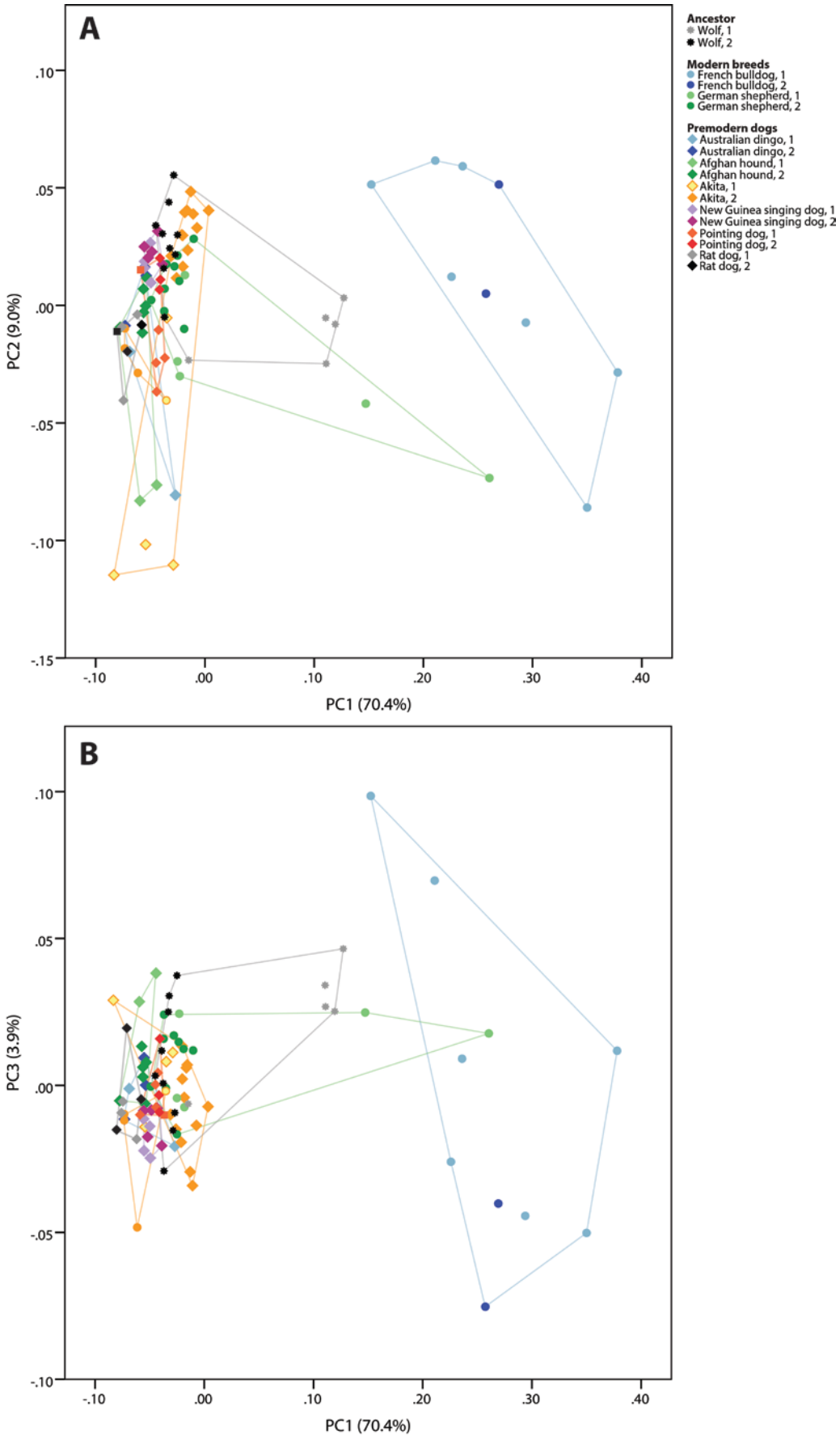

Figure S5. Principal component analysis of mandibular shape comparing the ontogenetic series of the wolf the ontogenetic series of different groups of domestic dogs.

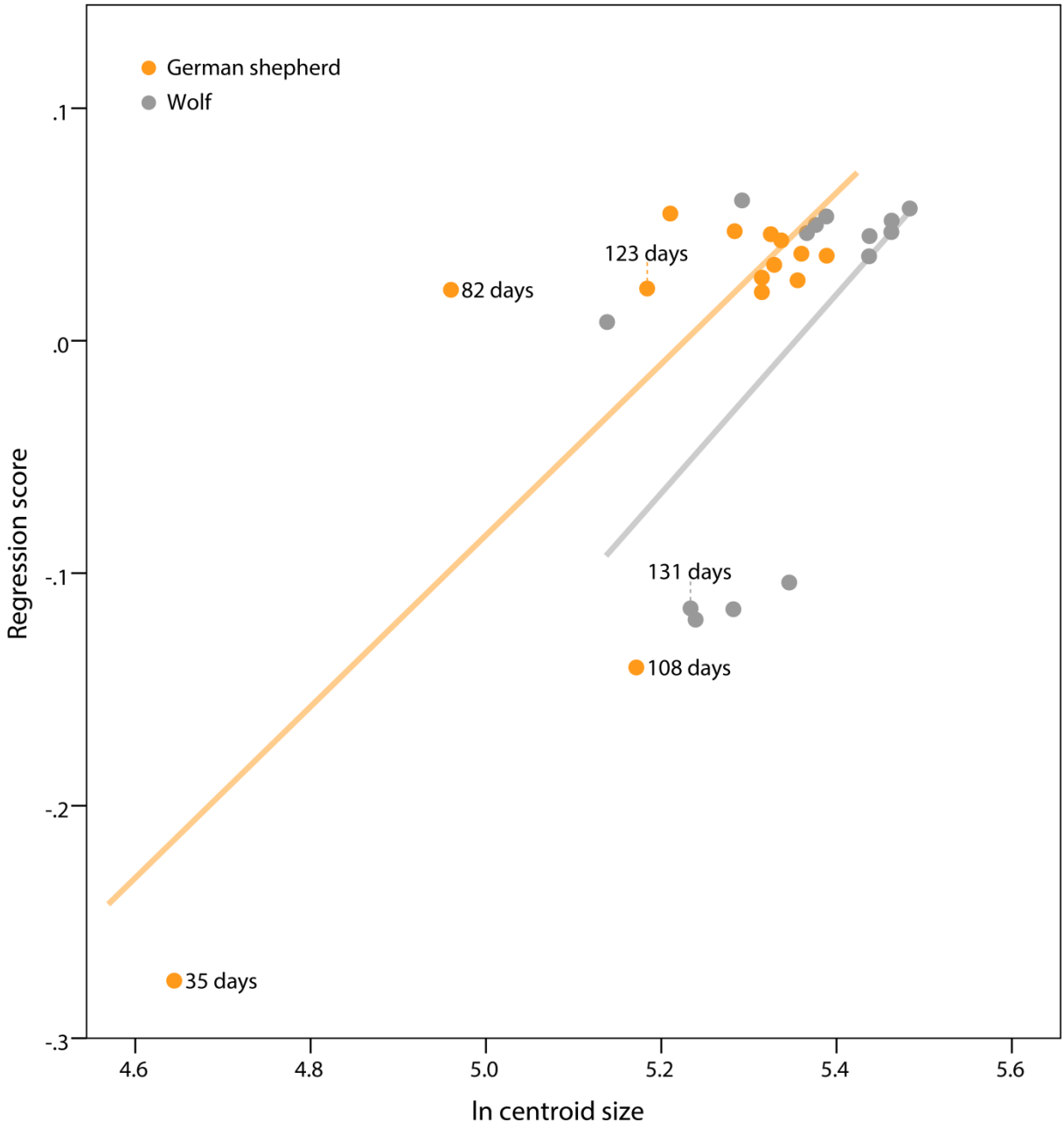

Figure S6. Ontogenetic trajectories of the German shepherd and the wolf in size-shape space. Numbers denominate the absolute age of the corresponding specimens (where known).

Table S1. Numbers and definition of cranial and mandibular landmarks used in the geometric morphometrics analyses. No., landmark number as depicted in Figure S1.

Cranial landmarks			
1	Interpremaxillary suture (dorsal), inferiormost point of the bony septum between the upper central incisors (dorsal)	18	Jugo-squamosal suture, inferior intersection, left side (dorsal)
2	Premaxillary-maxillary suture, inferiormost point, left side (dorsal)	19	Jugo-squamosal suture, inferior intersection, right side (dorsal)
3	Premaxillary-maxillary suture, inferiormost point, right side (dorsal)	20	Zygomatic process, highest projection, left side (dorsal)
4	Nasal bone, anterior tip, left side (dorsal)	21	Zygomatic process, highest projection, right side (dorsal)
5	Nasal bone, anterior tip, right side (dorsal)	22	Dorsal ethmoidal foramen, inferior margin left side (dorsal)
6	Intersection of nasal, maxillary, and frontal bones, left side (dorsal)	23	Dorsal ethmoidal foramen, inferior margin, right side (dorsal)
7	Intersection of nasal, maxillary, and frontal bones, right side (dorsal)	24	Interpremaxillary suture (ventral), anteriormost point of the bony septum between the upper central incisors (ventral)
8	Infraoribtal foramen, superiormost point, left side (dorsal)	25	Major palatine foramen, posterior margin, right side (ventral)
9	Infraoribtal foramen, superiormost point, right side (dorsal)	26	Major palatine foramen, posterior margin, left side (ventral)
10	Zygomatic process of maxilla, posterior tip, left side (dorsal)	27	Interpalatine suture, posteriormost point (ventral)
11	Zygomatic process of maxilla, posterior tip, right side (dorsal)	28	Presphenoid, anterior tip (ventral)
12	Fossa for lacrimal sac, inferior margin, left side (dorsal)	29	Palatine-pterygoid suture, inferiormost edge, right side (ventral)
13	Fossa for lacrimal sac, inferior margin, right side (dorsal)	30	Palatine-pterygoid suture, inferiormost edge, left side (ventral)
14	Zygomatic process of frontal bone, tip, right side (dorsal)	31	Hypoglossal canal, posterior margin, right side (ventral)
15	Zygomatic process of frontal bone, tip, right side (dorsal)	32	Hypoglossal canal, posterior margin, left side (ventral)
16	Bregma, intersection of interfrontal, interparietal, and frontoparietal sutures (dorsal)	33	Basion, ventral margin of the foramen magnum (ventral)
17	Inion, highest projection of the external occipital protuberance (dorsal)	34	Opisthion, dorsal margin of the foramen magnum (ventral)
Mandibular landmarks			
1	Mandibular symphysis, superiormost point between the lower central incisors	6	Mandibular notch, deepest point
2	Anterior mental foramen, posterior margin	7	Mandibular condyle, lateralmost point
3	Masseteric fossa, anterior tip	8	Condyloid crest, deepest point
4	Coronoid process, superiormost point	9	Angular process, posterior tip
5	Coronoid process, posteriormost point	10	Mandibular symphysis, posterior margin of the articular surface on the medioventral aspect of the dentary

Table S2. Number (n) of specimens examined per group.

Groups	Cranium		Mandible	
	n_{total}	$n_{juveniles}$	n_{total}	$n_{juveniles}$
Ancestor				
Wolf	14	5	14	5
Prehistoric dogs				
'Intermedius' type	1	0	1	0
'Palustris' type	5	0	1	0
Basal dogs				
Australian dingo	6	3	5	2
Afghan hound	8	2	8	2
Akita	15	4	16	4
New Guinea singing dog	9	4	9	4
Premodern dogs				
Pointing dog	8	5	8	5
Rat dog	6	3	6	3
Modern breeds				
French bulldog	9	7	9	7
German shepherd	14	5	14	5
Medium poodle	0	0	4	1
Miniature poodle	5	0	5	0
Standard poodle	5	0	5	0

Table S3. (separate excel file) Cranial and mandibular landmark coordinates from all investigated specimen. The first sheet (‘Replicates’) contains cranial and mandibular landmarks for every replicate (1 and 2) in all specimens; the second sheet (‘Means of replicates’) contains the averaged replicates from sheet1; the third sheet (‘estimated missing (cranium)’) contains all cranial landmarks including the estimated ones which were missing on the actual specimens. F, female; m, male; na and -9999, not applicable; 1, juvenile (permanent teeth not yet completely erupted); 2, adult (all permanent teeth completely erupted). Numbers of landmarks refer to numbers on Figure S1 and Table S1.

CONCLUSIONS AND PERSPECTIVES

Conclusions and perspectives

The investigation of ontogenetic processes can help to understand the generation of phenotypic disparity among and within clades as well as general processes related to evolution and domestication. The chapters of this thesis have tested several hypotheses dealing with one kind of developmental repatterning, which is heterochrony (Wallace, 2001).

The main focus was on different aspects of skeletal and dental growth, also in association with life history. First, I explored patterns of postnatal growth plate closure in relation to phylogenetic relatedness and locomotor adaptations in mammals (*Chapter 1*). Second, I investigated the timing of growth plate closure and tooth eruption in relation to sexual maturity in domestic dogs, trying to elucidate the influence of the domestication process on postnatal growth and life history (*Chapter 2*). Third, I investigated patterns of suture and synchondrosis closure in association with skull shape in domestic dogs as compared to human syndromes (*Chapter 3*). Fourth, I studied heterochronic processes and morphospace occupation of cranial and mandibular shape in domestic dogs (*Chapter 4*).

The work on growth plate closure in mammals (*Chapter 1*) is the first study synthesising a large amount of data on patterns of growth plate closure across mammals in a phylogenetic framework and could shed light on new and interesting aspects of the evolution of growth in different mammalian clades. This included the discovery of a common general pattern of growth plate closure sequence which is, however, not equal among species or higher-order taxa, although similarity has previously been hypothesised (Stevenson, 1924). Further, the observed patterns indicate that phylogenetic relatedness is influencing growth plate closure sequences more strongly than locomotor adaptations. We also found that heterochronic shifts in the growth plate closure sequence of marsupials occur with a higher rate than in placentals, which contrasts with the more limited variation in timing and morphospace occupation typical for marsupial development. Moreover, unlike placentals, marsupials maintain many epiphyses separated throughout life. This probably represents the derived state. Besides that, this study could provide useful information regarding the subsequent studies on domestic dogs: we found that most heterochronic shifts of the growth plate closure sequences across mammals occur in relatively young clades, i.e., 'families' and genera, and not in deep nodes, e.g., the marsupial-placental dichotomy. This result was promising regarding our studies within one single species (wolf and domestic dog) because it showed that heterochronic changes in markers of postnatal growth can to occur in closely related groups. Additionally, the work which is described in BOX 2 in the Introduction of this thesis showed that changes during postnatal growth are crucial for the generation of adult morphological disparity.

Chapter 2 gave evidence that the age at attainment of skeletal maturity in domestic dogs with relatively short legs (chondrodystrophic, e.g., dachshund) tends to be lower than in breeds with limb proportions typical for canids, e.g., poodle. *Chapter 3* further revealed that breeds with short and dorsally rotated rostra, e.g., French bulldog, exhibit more closed sutures and synchondroses than breeds with cranial proportions typical for canids, e.g., Bernese mountain dog. Both results emphasise that adult morphological disparity in domestic dogs correlates with heterochronic shifts in markers of skeletal growth. In the latter case, the causality of relationships remains elusive and must be subject of further studies on the nature of suture/synchondrosis closure. In other words, it is not clear from the here conducted study, whether alterations of skull shape throughout domestication, i.e., the artificial selection for dorsally rotated rostra in some breeds, have as a consequence the alteration of biomechanical forces acting on the skull. This in turn might have an impact on the here observed patterns of suture/

synchondrosis closure. As a second possibility, a short and dorsally rotated rostrum might be the direct result of a greater amount of suture/synchondrosis closure throughout the adult life of these dogs. This issue might best be tackled through the investigation of loading patterns on the skulls of bulldog-type domestic dogs as compared to loadings on the skulls of nonn-bulldog type breeds. Such studies could reveal how the highly derived short and dorsally rotated rostra with accompanying dental displacement and malocclusions influence loadings which act on the skull during biting and how these forces are distributed around the skull in cases where sutures/synchondrosis are open and closed. Similar studies have already been conducted in reptiles (Moazen et al., 2009; Curtis et al., 2013) but to my knowledge never in domestic dogs.

Concerning other aspects of postnatal growth, I did not detect heterochronic shifts. There are no shifts in the age at attainment of dental, skeletal and sexual maturity due to domestication. *Chapter 4* further revealed no heterochronic patterns during postnatal cranial and mandibular shape changes throughout the domestication process. These results emphasise that, although found as an underlying process or pattern in many biological systems, heterochrony is not omnipresent.

This thesis emphasizes the importance of carefully curated collections for comparative research, e.g., Museums, as most data came from collections in diverse institutions. However, despite the abundance of domestic dogs, skeletal material, especially of young individuals, is relatively scarce in collections. Moreover, certain breeds which would be most interesting to investigate in the context of the questions raised here (e.g., basset hound and corgi due to their chondrodystrophy and English bulldog due to its derived skull) are not often kept as pets and skeletons are therefore even scarcer. The usage of radiographs was a good alternative, but also here the gathering of large sample sizes was problematic. These circumstances led to not ideal sample sizes in *chapter 2* and *4*. Especially in *chapter 4*, the inclusion of juvenile prehistoric dogs would be extremely insightful for the investigation of growth trajectories throughout domestication, but archaeological remains of juvenile domestic dogs are rare due to their great porosity and greater proneness to decay (Brickley & Thomas, 2004). This last statement emphasises the importance of primary data. Future studies of domesticated mammal ontogeny would greatly benefit from efforts to increase samples of growth series of specific breeds of the animals of interest.

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APPENDIX

Growth trajectories in the cave bear and its extant relatives: an examination of ontogenetic patterns in phylogeny

This work was the MSc project of MF, who I was co-supervising during the second and third year of my PhD. MF collected the data. MF, MS and I conducted the analyses. MF wrote the manuscript and all authors then participated in discussing and drafting the final manuscript.

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Growth trajectories in the cave bear and its extant relatives: an examination of ontogenetic patterns in phylogeny

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Background
The study of postnatal ontogeny can provide insights into evolution by offering an understanding of how growth trajectories have evolved resulting in adult morphological disparity. The *Ursus* lineage comprises an ideal clade for studying cranial and mandibular shape and size variation in relation to postnatal ontogeny and phylogeny because it is at the same time not diverse but the species exhibit different feeding ecologies. *Ursus arctos* (brown bear), *U. maritimus* (polar bear), *U. americanus* (American black bear), and the extinct *U. spelaeus* (cave bear) were examined, using a three-dimensional geometric morphometric approach. Ontogenetic series of crania and mandibles ranging from newborns to senile age were sampled.

Results
The distribution of specimens in morphospace allowed to distinguish species and age classes and the ontogenetic trajectories were found to be more similar than expected by chance. Cranial shape changes during ontogeny are largely size related whereas the evolution of cranial shape disparity in this clade appears to be more influenced by dietary adaptation than by size and phylogeny. The different feeding ecologies are reflected in different cranial and mandibular shapes among species.

Conclusions
Shape changes detected between juvenile and adult skulls and mandibles in each taxon are concurrent with the cranial modifications required for the different feeding ecologies but do not correlate with important life history variables. The patterns of morphospace occupation of the cranium and the mandible in adults and through ontogeny are different.

Keywords: Mammalia, *Ursus*, ontogeny, variation, disparity, evolution, morphometrics, cranium, mandible

Background

Understanding the evolution of skull shapes by examining skull growth trajectories of related species helps to understand the modifications of cranial structures that arise in relation to diet, size, and phylogeny. Growth trajectories of skull shape have been studied in diverse vertebrate groups such as in, among many other taxa, Triturus newts [1], Podarcis lizards [2], chelid turtles [3], Caiman species [4], avian and non-avian dinosaurs [5], the rodents Sigmodon fulviventer [6] and Thrichomys apereoides [7], the spotted hyena (Crocuta crocuta) [8], the felids Puma concolor [9], Herpailurus yagouaroundi, and Acinonyx jubatus [10], the canid Lycalopex culpaeus [11], the common and pigmy Hippopotamus amphibious and Hexaprotodon liberiensis, respectively [12], the hominins Pan paniscus and Pan troglodytes [13], Neanderthals [14], and modern humans [15]. Findings in canids are that size and shape stand in relation to dietary shift after weaning [11]; in hyanids, skull size and shape maturity precedes sexual ma-

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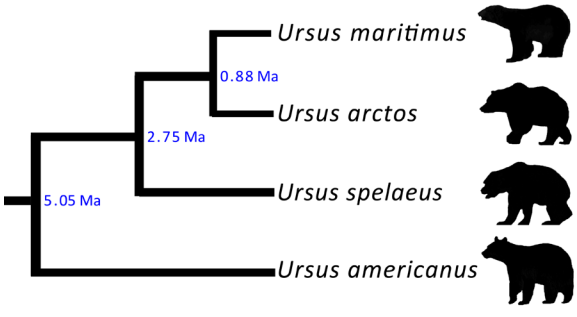
turity due to strong competition for food [8]; in felids, ontogenetic shape change is due to size and is not constrained by phylogeny [10]. All in all, investigated clades seem to express different patterns of skull shape trajectories and its correlates. More studies, including more and also fossil taxa are needed to further enlightening patterns of cranial shape change during ontogeny and across evolutionary time scales.

Geometric morphometric (GM) studies on ontogenetic trajectories were already conducted on diverse groups of carnivorans. In this study three-dimensional (3D) GM will be applied for the first time on species of the genus *Ursus*, a lineage that is not particularly diverse and contains herbivorous, omnivorous, and carnivorous species. This clearly constitutes an advantage of the present study, as shape change among relatively few, closely related species and these feeding ecologies can be investigated. The extant *Ursus* lineage includes *U. arctos* (brown bear), *U. maritimus* (polar bear), *U. americanus* (American black bear), and U. thibetanus (Asiatic black bear). Several aspects of the timing and patterns of divergence of ursid species have been investigated in recent years [16-24] (Fig. 1). We investigate intra- and interspecific cranial shape changes in extant *U. arctos*, *U. maritimus*, and *U. americanus* and compare the shape changes and life history traits to the extinct relative, *U. spelaeus* (cave bear), in order to trace and understand the evolutionary skull shape change. Because different diets demand specific adaptations of the jaw musculature and skull shape, it is expected to detect shape modifications associated with dietary changes among adults of different species and changes from sub-adult period to adulthood. Life-history information of *U. spelaeus* and of the extant species examined is listed in Figure 1. Although fossils harbour the difficulties of incomplete and insufficient sampling, it is worth to include them into a study of skull growth trajectories, since they, even if fragmentary, shed light on the evolution of observed changes and the generation of phenotypic disparity that can be observed at the present day.

U. spelaeus appeared around 126,000 years before present, at the beginning of the last interglacial [25] and became extinct during the last glacial-interglacial cycle around 27,800 years before present [26]. Innumerable remains of *U. spelaeus* have been found in caves throughout Europe, which have accumulated over a period of thousands of years. Not only the quantity of the remains but also their well-preserved condition makes this species a particularly valuable object for paleontological research. It provides a unique possibility to examine the morphological variation [27], feeding ecology [28,29], and ontogeny [30,31] of this species together with its



	Birth Weight (g)	Weaning Time (months)	Sexual Maturity (years)	Adult Weight (kg)	Diet
Ursus maritimus	600[1]	24-28[1]	5-6[1]	300-800 (m)[1] 150-300 (f)	carnivorous[1]
Ursus arctos	340-680[2]	18-30[2]	4-6[2]	80-600[2]	omnivorous[2]
Ursus spelaeus	-	-	-	400-500 (m)[3] 225-250 (f)	herbivorous/ omnivorous[4]
Ursus americanus	220-450[2]	6-8[2]	2-9[2]	47-409 (m)[2] 39-236 (f)	omnivorous[2]

Figure 1. Phylogenetic tree and life history data of the investigated species. The divergence times are based on molecular data suggested by Krause et al. [16]. Life history data were taken from the literature: 1, De Maseter & Stirling [56]; 2, Myers et al. [49]; 3, Christiansen [57]; 4, Bocherens et al. [58], Hilderbrand et al. [52], Stiner et al. [59], Richards et al. [51], Figueirido et al. [29]. M, male; f, female.

extant relatives. Previous studies about cranial shape variation in *U. spelaeus* focused on intraspecific variation [27] and ecomorphology [28,29,32,33]. A strong correlation between feeding ecology and craniodental morphology in the Ursidae lineage was reported by Sacco & Van Valkenburgh [34] and Christiansen [28]. However, there are only few studies on patterns of ontogeny of *U. spelaeus* crania in comparison to extant relatives. In a detailed study, Ehrenberg [30] described and compared a neonate *U. spelaeus* specimen with a *U. arctos* neonate as well as the ontogeny of these two species. Another work focused on dental eruption in *U. spelaeus* and *U. arctos* [35].

3D GM and multivariate analyses are used to assess and compare the extent of overlap or dissociation of extinct (*U. spelaeus*) and extant bear (*U. arctos*, *U. maritimus*, *U. americanus*) skull shapes in morphospace. The cranial and mandibular growth of the *Ursus* lineage is approached in a comparative perspective, considering the morphological and ecological diversity of this clade. The following questions will be addressed: a) are ontogenetic trajectories conserved during evolution, b) are they constrained by phylogeny, c) is intraspecific skull shape variation merely due to size, d) how do skull shapes change during ontogeny?

Methods

Specimens

A total of 256 crania and 186 mandibles of the extinct *U. spelaeus* and the extant species *U. arctos*, *U. maritimus*, and *U. americanus*, were investigated (Table 1). The sample consisted of juveniles and adults, including both sexes. None of the specimens appeared to have exhibited pathologies that affected skull shape.

The investigated crania are housed in the collections of several institutions in Europe: IfPEN, Institut für Paläontologie Erlangen, Erlangen, Germany; IPUW, Institut für Paläontologie Wien, Vienna, Austria; MCP, Château de Montbéliard, Montbéliard, France; NHMW, Naturhistorisches Museum Wien, Vienna, Austria; NMB, Naturhistorisches Museum Basel, Basel, Switzerland; NMBE, Naturhistorisches Museum Bern, Berne, Switzerland; NMSG, Naturmuseum St. Gallen, St. Gallen, Switzerland; RBIN, Institut Royal des Sciences Naturelles de Belgique, Brussels, Belgium; ZMUZH, Zoologisches Museum der Universität Zürich, Zurich, Switzerland. The specimens of *U. spelaeus* have been found in various caves in Central Europe and the Middle East: Gondenans-les-Moulins, Goyet Cave, Zoolithenhöhle, Mixnitz, Salzhofenhöhle, Sloupa, Kiriteinenhöhle, Conturines, Ramesch, Schwabenreith. More detailed information of the sample can be found in Table A1. Unless not stated otherwise, all analyses were performed using MorphoJ 1.06b, an integrated software package for geometric morphometrics [36].

Age and sex determination

The examined specimens were classified as juvenile, corresponding to IDAS 1 and 2 (permanent dentition not yet completed), or adult, corresponding to IDAS 3 to 5 (completed eruption of permanent dentition) [37]. Sexual dimorphism in the *Ursus* lineage is primarily expressed through a larger body size of adult males compared to adult females as well as through differences in total size of the canine teeth [28,38-40]. No sexual dimorphism is generally apparent in juveniles [31,40]. Consistent sexing of U. speleaus and extant bear species of unknown sex according to dental measurements described by Gordon & Morejohn [40] was not feasible because of great adult size variation among specimens from different populations. This issue has already been noted by Gordon & Morejohn [40]. Therefore, shape and size differences based on sexual dimorphism were not considered in this study.

Landmarks and data analysis

Sixty-one landmarks describe both lateral

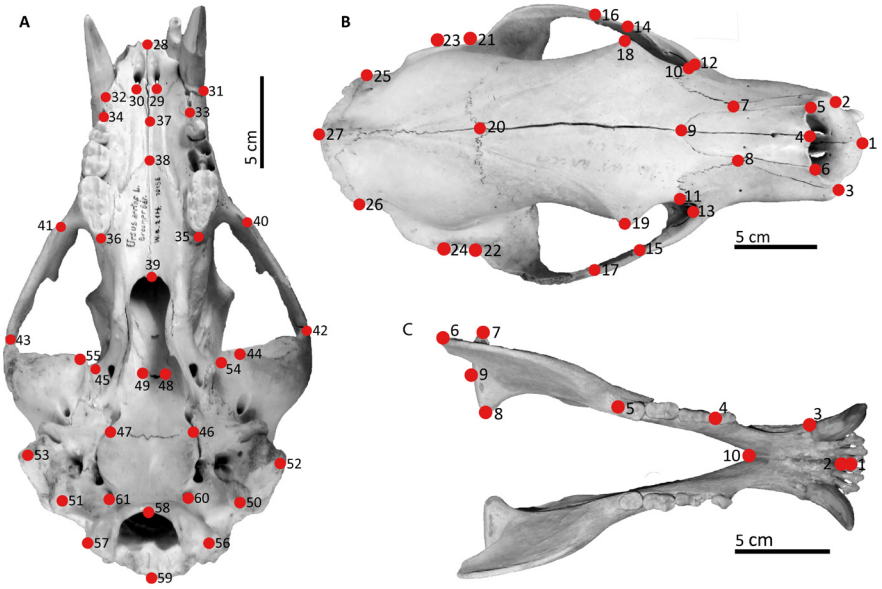


Figure 2. Cranial landmarks used in the morphometric analysis. Landmarks are illustrated on *U. arctos* ZMUZH10158 in ventral (A) and dorsal (B) aspects. Mandibular landmark positions illustrated on *U. maritimus* BA.3270 in dorsal aspect (C).

sides of the cranium and ten landmarks describe one side of the mandible (Table 2, Fig 2). The landmarks represent homologous structures that are clearly recognisable in every age stage and in all four species examined. To digitize the skulls in three dimensions, a MicroScribe MX 3D digitizer (Solution Technologies, Inc.) with 5 degrees of freedom and an accuracy of 0.1016 mm was used.

In some fossil *U. spelaeus* specimens certain cranial landmarks are missing. These missing landmarks were extrapolated with the R-function "estimate.missing" implemented in the R package "geomorph" v.2.1 [41]. The landmark data of all crania and mandibles were superimposed using Generalized Procrustes analysis [42-44]. This method optimally translates and rotates the homologous landmarks and scales them to the unit centroid size to minimize the difference between landmark configurations, which makes it easier to compare the shape of different objects. Because both sides of the cranium were used, only the symmetric components of the averaged (left and right sides of the skull) landmarks were used for calculating a covariance matrix and subsequent multivariate analyses. A principal component analysis (PCA) including all four species was performed. The PCA identifies patterns of variation and covariation of the landmark configurations and simplifies them by replacing the original variables with new ones (principal components, PCs) representing major axes of variance. PCs are linear

Table 1. Number (n) of specimens examined in this study.

Species	n (cranium)			n (mandible)		
	Adults	Juveniles	Total	Adults	Juveniles	Total
U. arctos	56	27	83	56	21	77
U. americanus	28	2	30	28	2	30
U. maritimus	42	1	43	43	1	44
U. spelaeus	97	3	100	31	4	35

Table 2 (next page). List of cranial and mandibular landmarks and their definition used in this study (Fig. 2).

Cranial landmarks (dorsal)	
1	Anterior point of the interpremaxillary suture at the alveolar margin of the incisors
2/3	Anterior point of the premaxillo-maxillary suture at the alveolar margin of the incisors
4	Anterior point of the internasal suture
5/6	Anterior point of the premaxillo-nasal suture
7/8	Point where frontal bone, premaxilla, and maxilla meet
9	Intersection of internasal and interfrontal sutures
10/11	Dorsal point of the lacrimal bone where it meets the frontal bone and the maxilla
12/13	Anterodorsal point of the jugal bone where it meets the maxilla
14/15	Dorsal t ip of the frontal process at the zygomatic arch
16/17	Dorsal point of the jugo-squamosal suture
18/19	Tip of the post-orbital process
20	Intersection of the interparietal and interfrontal sutures
21/22	Anterior point of the external auditory meatus
23/24	Posterior point of the external auditory meatus
25/26	Intersection of parietal, squamosal, and supraoccipital bones
27	Distal point of the external occipital protuberance
Cranial landmarks (ventrall)	
28	Posterior point of the interpremacillary suture at the alveolar margin of the incisors
29/30	Posterior point of the incisive foramen
31/32	Posterior point of the canine alveolus
33/34	Anterior point of the alveolar margin of P4
35/36	Posterior point of the alveolar margin of the tooth row
37	Intersection of interpremaxillary and intermaxillary sutures
38	Intersection of intermaxillary and interpalatine sutures
39	Posterior point of maximum concavity on the palatine
40/41	Ventral point of the jugo-maxillary suture
42/43	Ventral point of the jugo-squamosal suture
44/45	Anterior point of the alisphenoid-squamosal suture
46/47	Intersection of basioccipital, basisphenoid, and auditory bulla
48/49	Intersection of basisphenoid, presphenoid, and pterygoid suture
50/51	Ventral tip of the jugular process
52/53	Tip of the mastoid process
54/55	Ventral tip of the postglenoid process
56/57	Lateral point of the occipital condyle
58	Antero-ventral point of the foramen magnum
59	Postero-dorsal point of the foramen magnum
60/61	Central point of the hypoglossal foramen
Mandible landmarks	
1	Antero-ventral point of the mandibular symphysis and anterior part of the alveolar margin of the incisor
2	Antero-dorsal point of the mandibular symphysis and posterior part of the alveolar margin of the incisor
3	Postero-dorsal border of the canine alveolus
4	Anterior point of the alveolar margin of p4
5	Posterior point of the alveolar margin of the tooth row
6	Posterior edge of the coronoid process
7	Lateral edge of the articular surface of the condyloid process
8	Medial edge of the articular surface of the condyloid process
9	Tip of the angular process
10	Ventral point of the symphyseal region

combinations of the original variables and independent of each other [45]. Shape changes along the *Ursus* lineage can thus be visualized in a morphospace, investigating the position of taxa along those major axes of variance. The PCA was used to study the distribution of the specimens considering different species and age classes in morphospace.

Cranial shape changes in relation to size. To investigate the extent to which the shape variation is associated with size, regression analyses of the Procrustes coordinates onto log centroid size was performed. Centroid size, used as an estimate for skull size, is defined as the squared root of the sum of squared distances of each landmark from the centroid of the landmark configuration [45,46]. In this way the effect of allometry was eliminated. PCA were then conducted on the regression residuals. For comparison of the size associated shape modifications of the different species, the angles between the corresponding regression vectors were calculated and similarity between them was tested as described by Drake & Klingenberg [47].

Age stage and species differentiation. To calculate Procrustes distances between species and age stages, canonical variates analyses were conducted for cranial and mandibular landmarks, using ontogenetic stages (two groups) and species (four groups) as classifier variables. Procrustes distances were calculated based on Procrustes coordinates and on regression residuals of Procrustes coordinates (to remove the effect of allometry within species), respectively. The significance of the group differences was tested with permutation tests using 10,000 resamples.

Phylogenetic comparison. By mapping known phylogenetic trees onto size corrected PC scores [16], the effect of phylogeny on shape changes was investigated. In other words, we investigated whether similarity in shape correlates with phylogenetic relatedness. The phylogenetic signal was tested simulating the null hypothesis of the complete absence of a phylogenetic signal by randomly permuting the phenotypic data using 10,000 iterations [48]. For this purpose, the total amount of squared change, summed over all branches of the tree was used. The analysis was performed once based on the divergence times suggested by Krause et al. [16] and once using a phylogenetic tree with branch lengths set as equal to one for all taxa. Only PC scores of adult specimens were included in this analysis and branches were not weighted.

Results

Shape variation in cranial morphology

The first three principal components of cranial shape across species account for 58.47% of the total variation. PC1 is associated with a set of transformations that separates the juveniles from their adult conspecifics (Fig. 3a & A1). Juvenile specimens are characterised by a relatively short and wide face and palate as well as a rounded skull with a high and domed braincase (Fig. 3a). In addition, the zygomatic arches are relatively contracted and the foramen magnum is ventrally oriented. Along PC1, from negative to positive values, the whole cranium elongates, the braincase flattens and the zygomatic arches expand. Especially the rostrum gets relatively long and narrow. The foramen magnum shifts to a more posterior position and the prebasial angle is more declined (ventrally rotated) (Fig. 3a). PC1 further separates adult *U. spelaeus* from the adults of the other species. PC2 distinguishes some *U. spelaeus* and *U. arctos* juveniles from the adult conspecifics and the adult *U. spelaeus* from the adult *U. americanus* and *U. maritimus* (Fig. 3a). *U. arctos* occupies a wide range along PC2 so that the adult specimens overlap with the other species. Adult *U. maritimus* exhibit a relatively plane and narrow cranial shape compared to all other groups. The braincase is usually relatively flat and long and the internasal suture (Landmark 4) is shifted rostrally, leading to long nasal region (Fig. 3a). On the contrary, the crania of adult *U. spelaeus* as well as some

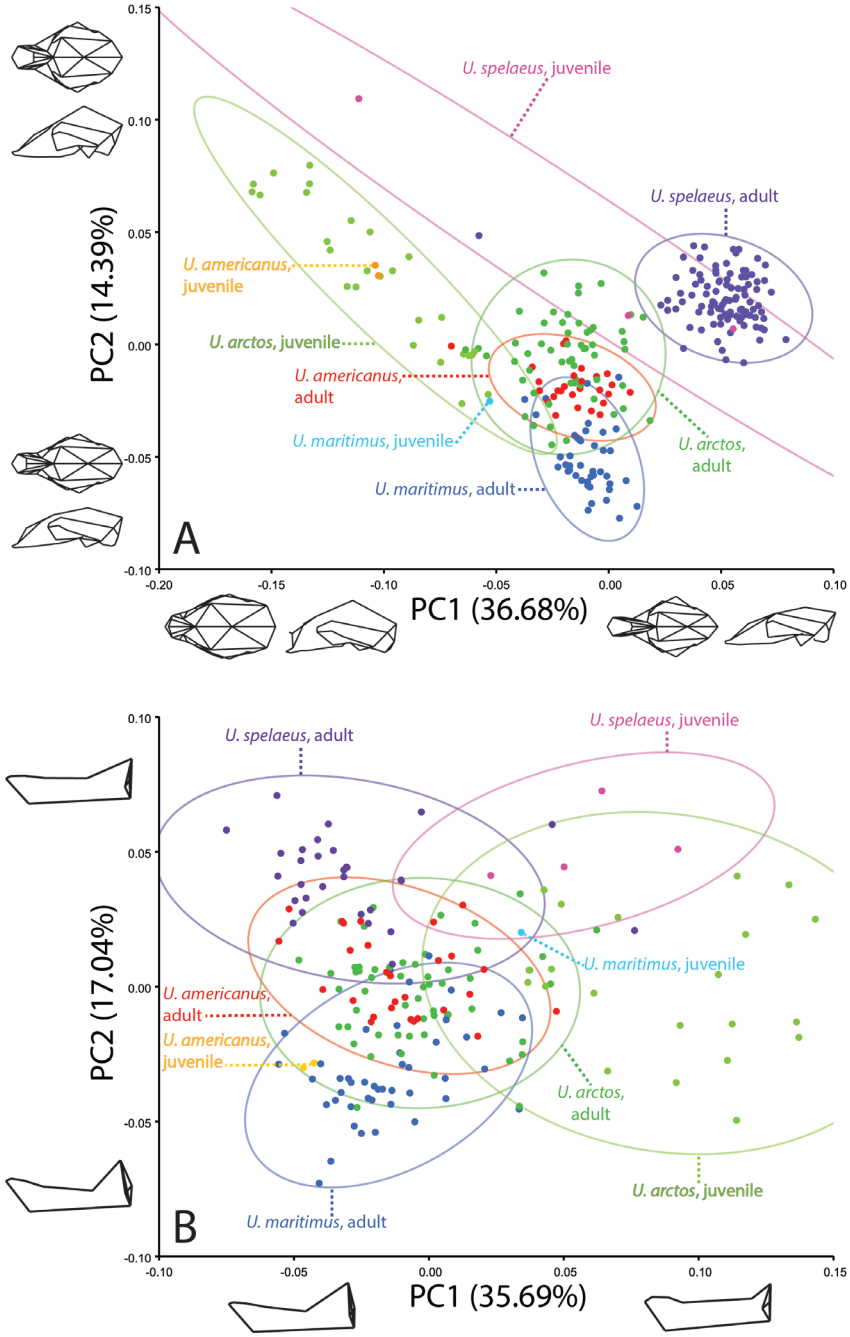


Figure 3. Principal component analysis of cranial (A) and mandibular (B) shape variation in juvenile and adult bear species. Ellipses represent the 95% confidence interval of the age stages (juvenile and adult) within species. Cranial and mandibular models represent extreme shape on PC1 and PC2.

juveniles of *U. spelaeus* and *U. arctos* have a relatively shorter and broader braincase with high vaulted calvaria. The nasals and palates are also relatively shorter and wider. An intermediate state can be observed in *U. arctos* and *U. americanus*. Unlike the first two principal components, PC3 does not distinguish between species or age stages (Fig. A1a).

To summarise, along PC1 the shape change from juvenile to adult specimens, regardless of species, can be traced, which is mainly reflected in a shape change from a relatively short face and high crowned braincase to a relatively more elongated and flattened braincase, long face, and expanded zygomatic arches. Looking at the adult specimens only, PC1

as well as PC2 separate *U. spelaeus* from the extant taxa, with the first having a relatively more domed braincase and shorter nasals than the extant species. The adults of the extant taxa exhibit an overlap in morphospace but can be separated to some extent along PC2. Visual examination of the shape space reveals a parallel course of the ontogenetic trajectories of *U. spelaeus* and *U. arctos* (Fig. 3a).

Computing Procrustes distances between juveniles and adults of the species analysed revealed that the species are significantly different from one another when considering only the adults (Table 3). This is not the case in the juveniles, which exhibit similar Procrustes distances between the species, except for a significant difference between juvenile *U. arctos* and *U. spelaeus* (Table 3). Moreover, the juvenile specimens are significantly different from their adult conspecifics (Table 3).

Shape variation in mandibular morphology

The first three principal components of mandibular shape space account for 62.0% of the total variation. The PCA of the mandibular landmarks presents a different distribution of the species and age classes in morphospace

Table 3. Cranial and mandibular Procrustes distances between age classes and species. Procrustes distances for non-size corrected and size corrected variation is given. Significance values are given in brackets and significant results are in bold. Comparisons between the species considering adults and juveniles are in dark grey light grey, respectively.

		U. americanus Adult	*U. arctos* Adult	*U. maritimus* Adult	*U. spelaeus* Juvenile	*U. americanus* Juvenile	*U. arctos* Juvenile	*U. maritimus* Juvenile
(a) Procrustes shape variation of the cranium								
U. arctos	Adult	**0.0466 (<.0001)**						
U. maritimus	Adult	**0.0534 (<.0001)**	**0.066 (<.0001)**					
U. spelaeus	Adult	**0.0846 (<.0001)**	**0.081 (<.0001)**	**0.0968 (<.0001)**				
U. americanus	Juvenile	**0.1232 (0.0025)**	**0.1237 (0.0004)**	**0.1416 (0.0009)**	**0.1673 (0.0001)**			
U. arctos	Juvenile	**0.1083 (<.0001)**	**0.1018 (<.0001)**	**0.1281 (<.0001)**	**0.1557 (<.0001)**	0.0623 (0.4594)		
U. maritimus	Juvenile	**0.0781 (0.0466)**	0.0817 (0.086)	**0.0767 (0.0226)**	**0.1253 (0.0214)**	0.119 (0.3308)	0.0916 (0.4181)	
U. spelaeus	Juvenile	**0.0781 (0.0117)**	**0.0724 (0.0477)**	**0.1041 (<.0001)**	**0.0804 (0.0103)**	0.1218 (0.3257)	**0.0988 (0.025)**	0.0986 (0.7511)
(b) Nonallometric shape variation of the cranium								
U. arctos	Adult	**0.0521 (<.0001)**						
U. maritimus	Adult	**0.0542 (<.0001)**	**0.0661 (<.0001)**					
U. spelaeus	Adult	**0.0556 (<.0001)**	**0.0584 (<.0001)**	**0.0829 (<.0001)**				
U. americanus	Juvenile	**0.0849 (0.002)**	**0.0819 (0.0379)**	**0.0983 (0.00 04)**	**0.0667 (0.0041)**			
U. arctos	Juvenile	**0.0625 (<.0001)**	**0.0436 (<.0001)**	**0.0785 (<.0001)**	**0.0303 (<.0001)**	0.0623 (0.4379)		
U. maritimus	Juvenile	0.072 (0.0694)	0.0727 (0.1605)	0.061 (0.142)	**0.0792 (0.0287)**	0.1054 (0.3379)	0.0736 (0.5715)	
U. spelaeus	Juvenile	**0.0819 (0.0002)**	**0.0869 (0.0023)**	**0.1101 (<.0001)**	0.0457 (0.0585)	0.0975 (0.2958)	0.0676 (0.1449)	0.104 (0.4083)

Table 3 (cont.)

		U. americanus Adult	*U. arctos* Adult	*U. maritimus* Adult	*U. spelaeus* Juvenile	*U. americanus* Juvenile	*U. arctos* Juvenile	*U. maritimus*
(a) Procrustes shape variation of the mandible								
U. arctos	Adult	**0.0292 (<.0001)**						
U. maritimus	Adult	**0.0444 (<.0001)**	**0.039 (<.0001)**					
U. spelaeus	Adult	**0.0508 (<.0001)**	**0.0535 (<.0001)**	**0.0753 (<.0001)**				
U. americanus	Juvenile	**0.0927 (0.0002)**	**0.0956 (<.0001)**	**0.0842 (0.0005)**	**0.1055 (0.0025)**			
U. arctos	Juvenile	**0.1048 (<.0001)**	**0.0952 (<.0001)**	**0.1115 (<.0001)**	**0.1251 (<.0001)**	**0.1579 (0.0034)**		
U. maritimus	Juvenile	0.0717 (0.1428)	0.0756 (0.0668)	**0.0882 (0.0176)**	0.0885 (0.1208)	0.1327 (0.3341)	0.0842 (0.3125)	
U. spelaeus	Juvenile	**0.0967 (<.0001)**	**0.0914 (<.0001)**	**0.1173 (<.0001)**	**0.0943 (0.0002)**	0.1488 (0.0643)	**0.0707 (0.0147)**	0.0838 (0.6031)
(b) Nonallometric shape variation of the mandible								
U. arctos	Adult	**0.0384 (<.0001)**						
U. maritimus	Adult	**0.0481 (<.0001)**	**0.0394 (<.0001)**					
U. spelaeus	Adult	**0.0492 (<.0001)**	**0.043 (<.0001)**	**0.0705 (<.0001)**				
U. americanus	Juvenile	**0.1154 (0.0017)**	**0.1339 (0.0004)**	**0.1214 (0.001)**	**0.1401 (0.0019)**			
U. arctos	Juvenile	**0.0653 (<.0001)**	**0.0402 (<.0001)**	**0.0668 (<.0001)**	**0.0405 (0.0003)**	**0.1642 (0.0013)**		
U. maritimus	Juvenile	0.0658 (0.1276)	0.0689 (0.0847)	**0.0806 (0.0171)**	0.062 (0.2214)	0.1542 (0.3295)	0.0657 (0.5905)	
U. spelaeus	Juvenile	**0.0798 (<.0001)**	**0.0745 (<.0001)**	**0.1021 (<.0001)**	0.0408 (0.1567)	0.1592 (0.1358)	0.0661 (0.0617)	0.0809 (0.7338)

(Fig. 3b) than the cranial analysis (Fig. 3a). PC1 displays a slight gradient from juveniles (positive values) to adults (negative values) in *U. spelaeus*, *U. arctos*, and *U. maritimus*, although overlap of the confidence ellipses is extensive (Fig. 3b). The mandibular shape modification from juvenile to adult is generally characterised by the relative enlargement of the coronoid process, the reduction of tooth row length (p4 to m3) in relation to the whole mandible, and the change in the angle between the buccal and labial part of the mandible. PC2 separates adult *U. maritimus* (negative values) from *U. spelaeus* (positive values) (Fig. 3b). In contrast to *U. spelaeus*, *U. maritimus* features relatively large coronoid processes, long tooth rows (p4 to m3), and an enlarged anterior part of the mandible. *U. arctos* and *U. americanus* exhibit an intermediate shape (centred around 0). PC3 does not separate species or age stages (Fig. A1b).

The comparison of mandibular Procrustes distances across the species and age stages revealed similar results as in the cranium. The adults from the different species are significantly different from each other and all juveniles are different from their adult conspecifics (Table 3). Species cannot be separated if the juveniles are compared, except for significant differences between *U. arctos* and *U. americanus* as well as U. artcos and *U. spelaeus* (Table 3).

Although species and ontogenetic stages are better differentiated in the cranial shape space

than in the mandibular shape space (Fig. 3), both shape spaces exhibit ontogenetic modifications mainly along PC1. In both structures, the species are significantly distinguishable considering adults – whereas a distinction is not always possible looking at juveniles – and juveniles can be differentiated from their adult conspecifics (Table 3).

Cranial and mandibular shape changes in relation to size across ontog enetic stages

The multivariate regressions of cranial and mandibular shape on size across all species revealed a significant correlation in both cases ($p < 0.0001$). Size explains 14.45% of cranial and 21.9% of mandibular shape variation in *U. spelaeus*, 34.82% of cranial and 32.0% of mandibular shape variation in *U. arctos*, 20.89% of cranial and 29.7% of mandibular shape variation in *U. maritimus* and 30.33% of cranial and 15.4% of mandibular shape variation in *U. americanus*. Pairwise comparisons of species' ontogenetic trajectories that are reflected in cranial shape changes associated with size are more similar than chance in all comparisons (Table A2). Considering the mandible, however, the ontogenetic trajectory of *U. americanus* is different from all the other bear species (Table A2).

After removing the between-species effect of size on cranial shape, PC1 separates adult *U. spelaeus* (positive PC1 values) from adults of the extant species (mainly negative PC1 values) (Fig. A2), characterized by relatively expanded and more massive zygomatic arches, higher braincases, short nasals, and broad palates, from the recent species with smaller zygomatic arches, flatter braincases, and overall narrower skulls. Juveniles are no longer separated from their adult conspecifics considering the confidence ellipses alone (Fig. A2a). PC2 and PC3 do not separate species or age classes (Fig. A2a, b). Removing the effect of size on mandibular shape results in a lack of separation of species and age classes (Fig. A2c, d).

The visually observed lack of differentiation of species and age stages in the non-allometric cranial and mandibular shape space (Fig. A2) is only partially reflected in the Procrustes distances. Species can still be significantly distinguished from one another when considering adults, both in the cranial and mandibular shape space (Table 3). Differences between juveniles disappear in the non-allometric shape space, except for a persisting difference in the mandibular shape space between juvenile *U. arctos* and *U. americanus* (Table 3). The juvenile bears are also still significantly different from their adult conspecifics, except for *U. spelaeus* in cranial and mandibular shape spaces and *U. maritimus* in cranial shape space (Table 3).

Phylogenetic comparison

Permutation tests revealed that the null hypothesis of a lacking phylogenetic signal cannot be rejected: the p-values for cranial and mandibular data were non-significant, either when using branch length estimates from Krause et al. [16] ($p_{cranium} = 0.51$, $p_{mandible} = 0.16$) or branch lengths set equal to 1 ($p_{cranium} = 0.51$, $p_{mandible} = 0.17$).

Discussion

We explored the distribution of extinct *U. spelaeus* (cave bear) and three extant bear species *U. maritimus* (polar bear), *U. arctos* (brown bear), and *U. americanus* (black bear) in morphospace, investigating cranial and mandibular shape changes in ontogeny and adult shape changes among species. The cranial and mandibular shapes in adults of the different species can be significantly distinguished from the cranial and mandibular shapes in adults of the other species and from their juvenile conspecifics (Fig. 3a, Table 3), whereas the juveniles of different species often look similar and cannot be significantly differentiated (Table 3). Thus, most of the species specific (and feeding ecology related, see below) cranial and mandibular shape

appears only during postnatal growth. This comparison has to be interpreted with caution due to the relatively small sample size for juveniles. Further, there is an apparent parallel course of the ontogenetic trajectories of cranial shape in *U. spelaeus*, U. acrtos, and *U. americanus* (confirmed by similarity of angles between the ontogenetic trajectories), reflecting similar patterns of cranial growth in these species. The postnatal ontogenetic cranial and mandibular shape changes can be described as mainly size-dependent modifications along PC1, affecting especially the relative length, width, and height of the braincase, length and width of the rostrum, width of the zygomatic arches, and height of the coronoid process. These findings are consistent with quantitative descriptions in a study investigating *U. americanus* [38].

Dietary shifts in the course of the weaning period are probably not responsible for the significant differences of cranial shape between juveniles and adults. The weaning period in *U. arctos* ranges from 18 to 30 months (Fig. 1) and thus roughly corresponds with the age at which the adult dentition is completed in our sample of *U. arctos* (from one year of age onwards, Table A1). However, the cubs are already eating a variety of foods by about 5 months of age [49], an age at which the here examined *U. arctos* specimens do not yet have their complete adult dentition and are thus still categorised as juveniles (Table A1). Further, in *U. americanus*, the age at weaning is not correlated with the completion of the permanent dentition. While juvenile bears of about 1.5 years have been found to have their completed permanent dentition and are thus categorised here as adults [38], weaning is completed earlier, at six to eight months (Fig. 1). Similarly, the age at the attainment of sexual maturity in *U. arctos* and *U. americanus* is much later than the above reported age at completion of the adult dentition (Fig. 1). Therefore, neither the age at weaning, nor the age at attainment of sexual maturity appear to correlate with the observed cranial and mandibular shape changes between juveniles and adult conspecifics in these two extant bear species. We thus infer that probably also the extinct *U. spelaeus* did not exhibit cranial and mandibular shape changes associated with these life history variables.

Differences in cranial and mandibular shape that differentiate adult *U. spelaeus* from extant relatives are not dependent on phylogenetic relatedness and size (Fig. 3, A2) but could be related to diet. Hereby, PC2 might represent a gradient from carnivory (negative values) to herbivory (centred around 0) in both, cranial and mandibular shape spaces (Fig. 3), although overlapping is partially extensive. Non-parametric Kruskal-Wallis tests and post-hoc comparisons ("pgirmess" package version 1.5.9 [50] implemented in R) confirmed a significant difference of values for PC2 among the three dietary categories (herbivorous, omnivorous, carnivorous) in the cranial landmarks ($chi2 = 162.5$, $p < .00001$) and in the mandibular landmarks ($chi2 = 98.1$, $p < .00001$) as well as significant differences among all three groups ($p<0.05$). The carnivorous *U. maritimus* is characterised by a relatively long rostrum and flat braincase, whereas herbivorous *U. spelaeus* exhibit a relatively higher and wider braincase and a shorter rostrum. The omnivorous *U. americanus* occupies the same cranial and mandibular morphospace as the omnivorous *U. arctos* (Fig. 3). These findings are consistent with the results of Figueirido et al. [29], who investigated ecomorphologically correlated cranial and mandibular shape variation in ursines, finding shared craniodental traits, similar to ours, among herbivorous bear species and opposite features in carnivorous bears. Omnivorous bears showed intermediate craniodental morphology. However, since the allocation of *U. spelaeus* to an herbivorous diet has been debated [51,52], the allocation of PC2 to diet is arguable.

Many previous studies on skull morphometrics restricted themselves to either the cranium or the mandible as markers of morphological

change [10,12,27,31-33]. This is a reasonable approach, but it is clear that both parts document different degrees of complexity and can reveal different patterns, even if correlated [11,53]. Differences in the growth trajectories of the mandible and the cranium were found in this work, as had also been found in sabercats [54]. Independent inheritance of upper and lower jaw features have been reported in hybrids of different dog breeds [55]. Some descendants of such cross breeds inherit the muzzle and upper jaw of one parent and the lower jaw from the other. These features indicate the possible independence in the ontogenetic development of mandible and cranium. In this regard it would be valuable and appropriate not to consider the cranium and the mandible separately to deduce to the skull as a whole, but to investigate both structures.

Conclusions

The ontogenetic trajectories of the examined bear species are largely similar and cranial and mandibular shape changes during postnatal ontogeny, leading to species specific and feeding ecology related skull shape are largely size related. Age at weaning and attainment of sexual maturity are probably not related with shape differences between juveniles and adults. The evolution of cranial and mandibular shape disparity in this clade appears to be more influenced by dietary adaptation than by size and phylogeny. The distribution of specimens in morphospace allowed distinguishing species and age classes. The different feeding ecologies are reflected in different cranial and mandibular shapes among species. The separation of the ontogenetic stages within species and among the investigated species differs regarding mandible shape space and skull shape space. The more complex skull shape allowed for a more refined resolution in morphospace than the mandible.

Competing interests

The authors declare that there are non-financial competing interests (political, personal, religious, ideological, academic, intellectual, commercial or any other), no competing interests in the manuscript.

Authors' contributions

MF, MG, MRSV conceived and designed the study. MF carried out the sampling of the data. MF, MS carried out the data analysis. MF, MG, MS, MRSV carried out the interpretation, manuscript drafting, modification, and finalization. All authors read and approved the final manuscript.

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Supplementary information

Table A1. List of all examined crania and mandibulae. ID, collection number; IDAS, individual dental age stage [37]; sex, male (m) and female (f); absolute age is given in days (d), weeks (w), months (m), or years (y).

Structure	Collection	Species	ID	Absolute age	IDAS	Age class	Sex	Provenance
Cranium	NMB	*Ursus americanus*	BA-C.III.476	5/6 m	2	Juvenile		
Cranium	NMB	*Ursus americanus*	BA-C.III.477	5/6 m	2	Juvenile		
Cranium	NHMW	*Ursus americanus*	982		3	Adult		
Cranium	NHMW	*Ursus americanus*	8268		3	Adult		
Cranium	NHMW	*Ursus americanus*	8269		3	Adult		
Cranium	NHMW	*Ursus americanus*	8270		3	Adult		
Cranium	NHMW	*Ursus americanus*	8271		3	Adult		
Cranium	NHMW	*Ursus americanus*	8272		3	Adult		
Cranium	NHMW	*Ursus americanus*	8273		3	Adult		
Cranium	NHMW	*Ursus americanus*	8274		3	Adult		
Cranium	NHMW	*Ursus americanus*	8275		3	Adult		
Cranium	NHMW	*Ursus americanus*	40543		3	Adult		
Cranium	NHMW	*Ursus americanus*	63555		3	Adult		
Cranium	NHMW	*Ursus americanus*	64974		3	Adult		
Cranium	NMB	*Ursus americanus*	BA-8387		3	Adult	m	Zoo Basel
Cranium	NMB	*Ursus americanus*	BA-9151		3	Adult	f	Zoo Basel
Cranium	NMB	*Ursus americanus*	BA-9608	5 y	3	Adult	f	Zoo Basel
Cranium	NMB	*Ursus americanus*	BA-C.2189		3	Adult	f	Neufundland
Cranium	NMB	*Ursus americanus*	BA-C.III.475	5 y	3	Adult	f	
Cranium	NMBE	*Ursus americanus*	BE-1021778	5 y	3	Adult	m	Canada
Cranium	NMBE	*Ursus americanus*	BE-1042654	7 y	3	Adult	f	Canada
Cranium	NMBE	*Ursus americanus*	BE-1042659		3	Adult		USA
Cranium	RBIN	*Ursus americanus*	KBIN-1139		3	Adult	m	Zoo Brussels
Cranium	RBIN	*Ursus americanus*	KBIN-1139 B		3	Adult	f	Zoo Brussels
Cranium	RBIN	*Ursus americanus*	KBIN-3384	7 y	3	Adult	m	Solvay
Cranium	RBIN	*Ursus americanus*	KBIN-40169		3	Adult		
Cranium	RBIN	*Ursus americanus*	KBIN-40170		3	Adult		
Cranium	RBIN	*Ursus americanus*	KBIN-40171		3	Adult		
Cranium	RBIN	*Ursus americanus*	KBIN-40172		3	Adult		
Cranium	NMB	*Ursus americanus*	BA-2962	5 y	5	Adult	m	
Cranium	NHMW	*Ursus arctos*	983		1	Juvenile		
Cranium	NHMW	*Ursus arctos*	2579		1	Juvenile		
Cranium	NHMW	*Ursus arctos*	2580		1	Juvenile		
Cranium	NMB	*Ursus arctos*	BA-6363		1	Juvenile		Basel
Cranium	NMB	*Ursus arctos*	BA-C.3876		1	Juvenile	m	
Cranium	NMBE	*Ursus arctos*	BE-1042348	3 m	1	Juvenile	m	Tierpark Dhälhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042350	5 m	1	Juvenile		Tierpark Dhälhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042357	4 m	1	Juvenile	m	Tierpark Dhälhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042358	4 m	1	Juvenile	f	Tierpark Dhälhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042404	4 w	1	Juvenile		Tierpark Dhälhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042405	4 w	1	Juvenile		Tierpark Dhälhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042406		1	Juvenile	m	Tierpark Dhälhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042407		1	Juvenile	f	Tierpark Dhälhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042408	1.5 m	1	Juvenile	f	Tierpark Dhälhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042409	2 m	1	Juvenile	f	
Cranium	NMBE	*Ursus arctos*	BE-1042412	4 w	1	Juvenile		Tierpark Dhälhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042561	5 d	1	Juvenile		Tierpark Dhälhölzli Berne
Cranium	NHMW	*Ursus arctos*	21490		2	Juvenile		
Cranium	NMB	*Ursus arctos*	BA-8221	8 m	2	Juvenile		

Tabelle A1 cont.

Cranium	NMB	*Ursus arctos*	BA-C.III.351		2	Juvenile		
Cranium	NMBE	*Ursus arctos*	BE-1042347	6 m	2	Juvenile	m	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042349	6 m	2	Juvenile		Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042373	10 m	2	Juvenile	m	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042375	11 m	2	Juvenile	f	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042380	11 m	2	Juvenile	m	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042386	9 m	2	Juvenile	f	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042416	9 m	2	Juvenile	m	Tierpark Dählhölzli Berne
Cranium	NMB	*Ursus arctos*	BA-10823		3	Adult	f	Finland
Cranium	NMB	*Ursus arctos*	BA-6541		3	Adult		Berne
Cranium	NMB	*Ursus arctos*	BA-6542	12 m	3	Adult		Berne
Cranium	NMB	*Ursus arctos*	BA-C.1565		3	Adult	f	Berne
Cranium	NMB	*Ursus arctos*	BA-C.III.363		3	Adult	f	
Cranium	NMB	*Ursus arctos*	BA-C.III.999		3	Adult	m	
Cranium	NMBE	*Ursus arctos*	BE-1030174		3	Adult	f	
Cranium	NMBE	*Ursus arctos*	BE-1042355	2 y	3	Adult	f	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042361	2 y	3	Adult	f	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042363	3 y	3	Adult	f	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042364	3 y	3	Adult	m	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042369	5 y	3	Adult	m	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042372	2 y	3	Adult	f	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042376	5 y	3	Adult	m	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042379	11 y	3	Adult	m	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042381	2 y	3	Adult	m	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042383	3 y	3	Adult	m	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042384		3	Adult	f	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042387	1 y 1m	3	Adult	m	
Cranium	NMBE	*Ursus arctos*	BE-1042388	1.5 y	3	Adult	m	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042390	2.5 y	3	Adult	f	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042392		3	Adult	f	
Cranium	NMBE	*Ursus arctos*	BE-1042394	4 y	3	Adult	f	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042396	2 y	3	Adult	f	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042399		3	Adult	f	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042418		3	Adult	m	
Cranium	NMBE	*Ursus arctos*	BE-1042425	1.5 y	3	Adult	f	
Cranium	NMBE	*Ursus arctos*	BE-1042426	3 y	3	Adult	f	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042553		3	Adult	f	Menagerie
Cranium	NMBE	*Ursus arctos*	BE-1042556	5 y	3	Adult	f	Alaska
Cranium	NMBE	*Ursus arctos*	BE-1047854		3	Adult	m	Graubünden
Cranium	NMBE	*Ursus arctos*	BE-1054540		3	Adult	m	Alaska
Cranium	NMBE	*Ursus arctos*	BE-1054620		3	Adult	f	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1065825	5 y	3	Adult	m	Tierpark Dählhölzli Berne
Cranium	RBIN	*Ursus arctos*	KBIN-1136 C		3	Adult	f	Zoo Brussels
Cranium	ZMUZH	*Ursus arctos*	ZM10308		3	Adult		
Cranium	ZMUZH	*Ursus arctos*	ZM15198	2 y	3	Adult		
Cranium	NMB	*Ursus arctos*	BA-C.2470	8 y	4	Adult	m	North America
Cranium	NMBE	*Ursus arctos*	BE-1030173		4	Adult	m	Zoo Warsaw
Cranium	NMBE	*Ursus arctos*	BE-1030241	22 y	4	Adult	m	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042374	13 y	4	Adult	f	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042393	27 y	4	Adult	f	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042410	15 y	4	Adult	m	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042554		4	Adult		Canada
Cranium	NMBE	*Ursus arctos*	BE-1042343		5	Adult	m	Tierpark Dählhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042366	23 y	5	Adult	f	Hungary

Tabelle A1 cont.

Cranium	NMBE	*Ursus arctos*	BE-1042391	27 y	5	Adult	f	Romania
Cranium	NMBE	*Ursus arctos*	BE-1042395		5	Adult	m	
Cranium	NMBE	*Ursus arctos*	BE-1042398	30 y	5	Adult	m	Tierpark Dhälhölzli Berne
Cranium	NMBE	*Ursus arctos*	BE-1042413		5	Adult	m	
Cranium	NMBE	*Ursus arctos*	BE-1042414	32 y	5	Adult	m	Bosnia
Cranium	NMBE	*Ursus arctos*	BE-1042417		5	Adult	f	Zoo Warsaw
Cranium	NMBE	*Ursus arctos*	BE-1042555		5	Adult	m	Alaska
Cranium	NMBE	*Ursus arctos*	BE-1042557		5	Adult	m	Alaska
Cranium	NMBE	*Ursus arctos*	BE-1042563		5	Adult	f	Graubünden
Cranium	NMBE	*Ursus arctos*	BE-1054621		5	Adult	m	Mongolia
Cranium	NHMW	*Ursus maritimus*	22741		2	Juvenile		
Cranium	IPUW	*Ursus maritimus*	1322		3	Adult		
Cranium	IPUW	*Ursus maritimus*	1366		3	Adult		
Cranium	NHMW	*Ursus maritimus*	5321		3	Adult		
Cranium	NHMW	*Ursus maritimus*	7138		3	Adult		
Cranium	NHMW	*Ursus maritimus*	7140		3	Adult		
Cranium	NHMW	*Ursus maritimus*	7141		3	Adult		
Cranium	NHMW	*Ursus maritimus*	7142		3	Adult		
Cranium	NHMW	*Ursus maritimus*	7143		3	Adult		
Cranium	NHMW	*Ursus maritimus*	7144		3	Adult		
Cranium	NHMW	*Ursus maritimus*	7145		3	Adult		
Cranium	NHMW	*Ursus maritimus*	7147		3	Adult		
Cranium	NHMW	*Ursus maritimus*	7149		3	Adult		
Cranium	NHMW	*Ursus maritimus*	7225		3	Adult		
Cranium	NHMW	*Ursus maritimus*	7794		3	Adult		
Cranium	NHMW	*Ursus maritimus*	12691		3	Adult		
Cranium	NHMW	*Ursus maritimus*	13176		3	Adult		
Cranium	NHMW	*Ursus maritimus*	13329		3	Adult		
Cranium	NHMW	*Ursus maritimus*	31068		3	Adult		
Cranium	NMB	*Ursus maritimus*	BA-10286		3	Adult		Basel
Cranium	NMB	*Ursus maritimus*	BA-10302	8 y	3	Adult	f	Basel
Cranium	NMB	*Ursus maritimus*	BA-6548		3	Adult		
Cranium	NMB	*Ursus maritimus*	BA-8272		3	Adult		Greenland
Cranium	NMB	*Ursus maritimus*	BA-9985		3	Adult		
Cranium	NMB	*Ursus maritimus*	BA-C.1294		3	Adult	m	Basel
Cranium	NMB	*Ursus maritimus*	BA-C.3270	5 y	3	Adult		
Cranium	NMB	*Ursus maritimus*	BA-C.4045		3	Adult	m	
Cranium	NMB	*Ursus maritimus*	BA-C.III.146		3	Adult		
Cranium	NMB	*Ursus maritimus*	BA-n.N.039		3	Adult		
Cranium	NMBE	*Ursus maritimus*	BE-1049101		3	Adult	m	Greenland
Cranium	RBIN	*Ursus maritimus*	KBIN-1133 C		3	Adult	m	
Cranium	RBIN	*Ursus maritimus*	KBIN-1133 F		3	Adult	f	Zoo Brussels
Cranium	RBIN	*Ursus maritimus*	KBIN-1134		3	Adult	m	Zoo Brussels
Cranium	RBIN	*Ursus maritimus*	KBIN-1134 B		3	Adult	f	
Cranium	RBIN	*Ursus maritimus*	KBIN-7551		3	Adult	m	
Cranium	RBIN	*Ursus maritimus*	KBIN-7553		3	Adult	f	
Cranium	RBIN	*Ursus maritimus*	KBIN-7555		3	Adult	m	
Cranium	RBIN	*Ursus maritimus*	KBIN-7556		3	Adult	m	
Cranium	NMSG	*Ursus maritimus*	St.G1		3	Adult		
Cranium	NHMW	*Ursus maritimus*	2568		4	Adult		
Cranium	NMB	*Ursus maritimus*	BA-10247	8 y	4	Adult	m	Basel
Cranium	NMB	*Ursus maritimus*	BA-10248	8 y	4	Adult		
Cranium	NMBE	*Ursus maritimus*	BE-1042675		4	Adult	f	Zoo Basel
Cranium	Montbéliard	*Ursus spelaeus*	MCP-2003 01-137		1	Juvenile		Gondenans les Moulins

Tabelle A1 cont.

Cranium	NHMW	*Ursus spelaeus*	Juvenile		2	Juvenile		
Cranium	Montbéliard	*Ursus spelaeus*	MCP-2003 01 14		2	Juvenile	f	Gondenans les Moulins
Cranium	IPUW	*Ursus spelaeus*	45		3	Adult		Mixnitz
Cranium	NHMW	*Ursus spelaeus*	5325		3	Adult		Slouperhöhle
Cranium	NHMW	*Ursus spelaeus*	5334		3	Adult		
Cranium	IPUW	*Ursus spelaeus*	28.03.3000		3	Adult		Mixnitz
Cranium	NHMW	*Ursus spelaeus*	1907-71		3	Adult		
Cranium	IPUW	*Ursus spelaeus*	3000/3/43		3	Adult		Mixnitz
Cranium	IPUW	*Ursus spelaeus*	3000/5/103		3	Adult		Mixnitz
Cranium	IPUW	*Ursus spelaeus*	3000/5/104		3	Adult		Mixnitz
Cranium	IPUW	*Ursus spelaeus*	3000/5/105		3	Adult		Mixnitz
Cranium	IPUW	*Ursus spelaeus*	3003/3/39		3	Adult		Mixnitz
Cranium	NHMW	*Ursus spelaeus*	A5325		3	Adult		Slouperhöhle
Cranium	NHMW	*Ursus spelaeus*	A5327		3	Adult		Slouperhöhle
Cranium	NHMW	*Ursus spelaeus*	A5328		3	Adult		Slouperhöhle
Cranium	NHMW	*Ursus spelaeus*	A5330		3	Adult		Kreuzberghöhle
Cranium	NHMW	*Ursus spelaeus*	A5332		3	Adult		
Cranium	NHMW	*Ursus spelaeus*	A5543		3	Adult		Slouperhöhle
Cranium	NHMW	*Ursus spelaeus*	A5550		3	Adult		Slouperhöhle
Cranium	IPUW	*Ursus spelaeus*	Cu703		3	Adult		Conturines
Cranium	IPUW	*Ursus spelaeus*	Cu704		3	Adult		Conturines
Cranium	IfPEN	*Ursus spelaeus*	G/f1/1971/10		3	Adult		Zoolithenhöhle
Cranium	IfPEN	*Ursus spelaeus*	G/f1/1971/19		3	Adult		Zoolithenhöhle
Cranium	IfPEN	*Ursus spelaeus*	G/f1/1971/21		3	Adult		Zoolithenhöhle
Cranium	Montbéliard	*Ursus spelaeus*	MCP-2003 01-06		3	Adult	f	Gondenans les Moulins
Cranium	Montbéliard	*Ursus spelaeus*	MCP-2003 01-11		3	Adult		Gondenans les Moulins
Cranium	IfPEN	*Ursus spelaeus*	Mor2		3	Adult		Zoolithenhöhle
Cranium	RBIN	*Ursus spelaeus*	RBIN 2167		3	Adult	f	Goyet Cave B5
Cranium	RBIN	*Ursus spelaeus*	RBIN 2170		3	Adult	m	Goyet Cave B5
Cranium	RBIN	*Ursus spelaeus*	RBIN 2175		3	Adult	f	Goyet Cave B5
Cranium	IPUW	*Ursus spelaeus*	RK332		3	Adult		Ramesch
Cranium	Montbéliard	*Ursus spelaeus*	Ro85 518-19		3	Adult		Gondenans les Moulins
Cranium	IfPEN	*Ursus spelaeus*	sp.72/2		3	Adult		Zoolithenhöhle
Cranium	IfPEN	*Ursus spelaeus*	sp.72/4		3	Adult		Zoolithenhöhle
Cranium	IfPEN	*Ursus spelaeus*	sp.72/46		3	Adult		Zoolithenhöhle
Cranium	IfPEN	*Ursus spelaeus*	sp.72/49		3	Adult		Zoolithenhöhle
Cranium	IfPEN	*Ursus spelaeus*	sp.72/51		3	Adult		Zoolithenhöhle
Cranium	IfPEN	*Ursus spelaeus*	sp.72/58		3	Adult		Zoolithenhöhle
Cranium	IfPEN	*Ursus spelaeus*	sp.72/62		3	Adult		Zoolithenhöhle
Cranium	IfPEN	*Ursus spelaeus*	Sp.78/1		3	Adult		Zoolithenhöhle
Cranium	IPUW	*Ursus spelaeus*	Sr1/2		3	Adult		Mixnitz
Cranium	NMSG	*Ursus spelaeus*	St. G D24		3	Adult		Wildkirchlihöhle
Cranium	IPUW	*Ursus spelaeus*	SW185		3	Adult		Schwabenreith
Cranium	IPUW	*Ursus spelaeus*	SW2025		3	Adult		Schwabenreith
Cranium	IPUW	*Ursus spelaeus*	SW483		3	Adult		Schwabenreith
Cranium	IPUW	*Ursus spelaeus*	SW512		3	Adult		Schwabenreith
Cranium	IPUW	*Ursus spelaeus*	SW630		3	Adult		Schwabenreith
Cranium	IPUW	*Ursus spelaeus*	SW779		3	Adult		Schwabenreith
Cranium	IPUW	*Ursus spelaeus*	U50		3	Adult		Mixnitz
Cranium	NHMW	*Ursus spelaeus*	Unknown1		3	Adult		
Cranium	NHMW	*Ursus spelaeus*	Unknown3		3	Adult		Merkenstein
Cranium	IPUW	*Ursus spelaeus*	5		4	Adult		Mixnitz
Cranium	NHMW	*Ursus spelaeus*	22		4	Adult		
Cranium	IPUW	*Ursus spelaeus*	37		4	Adult		Mixnitz

Tabelle A1 cont.

Cranium	IPUW	*Ursus spelaeus*	70		4	Adult		Slouperhöhle
Cranium	NHMW	*Ursus spelaeus*	5317		4	Adult		
Cranium	NHMW	*Ursus spelaeus*	5338		4	Adult		
Cranium	NHMW	*Ursus spelaeus*	5557		4	Adult		Slouperhöhle
Cranium	IPUW	*Ursus spelaeus*	14,		4	Adult		Mixnitz
Cranium	IPUW	*Ursus spelaeus*	3000/3/37		4	Adult		Mixnitz
Cranium	IPUW	*Ursus spelaeus*	3000/5/106		4	Adult		Mixnitz
Cranium	NMB	*Ursus spelaeus*	BA-if.184		4	Adult	m	Gondenans les Moulins
Cranium	NMB	*Ursus spelaeus*	BA-jf.775		4	Adult	m	Gondenans les Moulins
Cranium	IfPEN	*Ursus spelaeus*	G/f1/1971/12		4	Adult		Zoolithenhöhle
Cranium	IfPEN	*Ursus spelaeus*	G/f1/1971/2		4	Adult		Zoolithenhöhle
Cranium	Montbéliard	*Ursus spelaeus*	MCP-2003 01-02		4	Adult		Gondenans les Moulins
Cranium	Montbéliard	*Ursus spelaeus*	MCP-2003 01-03		4	Adult	m	Gondenans les Moulins
Cranium	Montbéliard	*Ursus spelaeus*	MCP-2003 01-05		4	Adult		Gondenans les Moulins
Cranium	Montbéliard	*Ursus spelaeus*	MCP-2003 01-07		4	Adult		Gondenans les Moulins
Cranium	NMSG	*Ursus spelaeus*	St. G D13		4	Adult		Wildkirchlihöhle
Cranium	IPUW	*Ursus spelaeus*	SW1248		4	Adult		Schwabenreith
Cranium	IPUW	*Ursus spelaeus*	SW191		4	Adult		Schwabenreith
Cranium	IPUW	*Ursus spelaeus*	SW475		4	Adult		Schwabenreith
Cranium	IPUW	*Ursus spelaeus*	T25		4	Adult		Badlhöhle
Cranium	IPUW	*Ursus spelaeus*	U2		4	Adult		Kiriteinenhöhle
Cranium	NHMW	*Ursus spelaeus*	XXVII.3		4	Adult		Slouperhöhle
Cranium	NHMW	*Ursus spelaeus*	5544		5	Adult		Slouperhöhle
Cranium	NHMW	*Ursus spelaeus*	5545		5	Adult		Slouperhöhle
Cranium	IPUW	*Ursus spelaeus*	05.03.3000		5	Adult		Mixnitz
Cranium	IPUW	*Ursus spelaeus*	15.03.3000		5	Adult		Mixnitz
Cranium	IPUW	*Ursus spelaeus*	21.03.3000		5	Adult		Mixnitz
Cranium	IPUW	*Ursus spelaeus*	30.03.3000		5	Adult		Mixnitz
Cranium	IPUW	*Ursus spelaeus*	1,		5	Adult		Mixnitz
Cranium	IPUW	*Ursus spelaeus*	3000/5/108		5	Adult		Mixnitz
Cranium	IPUW	*Ursus spelaeus*	3000/5/109		5	Adult		Mixnitz
Cranium	IPUW	*Ursus spelaeus*	41,		5	Adult		Mixnitz
Cranium	NHMW	*Ursus spelaeus*	A5329		5	Adult		Slouperhöhle
Cranium	NHMW	*Ursus spelaeus*	A5552		5	Adult		Slouperhöhle
Cranium	NMB	*Ursus spelaeus*	BA-i65		5	Adult		Gondenans les Moulins
Cranium	IPUW	*Ursus spelaeus*	F65		5	Adult		Mixnitz
Cranium	Montbéliard	*Ursus spelaeus*	MCP-2003 01-01		5	Adult	m	Gondenans les Moulins
Cranium	Montbéliard	*Ursus spelaeus*	MCP-2003 01-04		5	Adult	m	Gondenans les Moulins
Cranium	IPUW	*Ursus spelaeus*	Mix-U1		5	Adult		Mixnitz
Cranium	RBIN	*Ursus spelaeus*	RBIN 2207		5	Adult	m	Goyet Cave B4
Cranium	NMSG	*Ursus spelaeus*	St. G D21		5	Adult	m	Wildkirchlihöhle
Cranium	NMSG	*Ursus spelaeus*	St.G 2		5	Adult	m	Wildkirchlihöhle
Cranium	NHMW	*Ursus spelaeus*	Unknown2		5	Adult		Bärenhöhle Hietlau
Cranium	NHMW	*Ursus spelaeus*	XXVII.2		5	Adult		Slouperhöhle
Cranium	NHMW	*Ursus spelaeus*	XXVII.4		5	Adult		Slouperhöhle
Mandible	NMB	*Ursus americanus*	BA-C.III.476	5/6 m	2	Juvenile		
Mandible	NMB	*Ursus americanus*	BA-C.III.477	5/6 m	2	Juvenile		
Mandible	NHMW	*Ursus americanus*	982		3	Adult		
Mandible	NHMW	*Ursus americanus*	8268		3	Adult		
Mandible	NHMW	*Ursus americanus*	8269		3	Adult		
Mandible	NHMW	*Ursus americanus*	8270		3	Adult		
Mandible	NHMW	*Ursus americanus*	8271		3	Adult		
Mandible	NHMW	*Ursus americanus*	8272		3	Adult		
Mandible	NHMW	*Ursus americanus*	8273		3	Adult		

Tabelle A1 cont.

Mandible	NHMW	*Ursus americanus*	8274		3	Adult		
Mandible	NHMW	*Ursus americanus*	8275		3	Adult		
Mandible	NHMW	*Ursus americanus*	40543		3	Adult		
Mandible	NHMW	*Ursus americanus*	63555		3	Adult		
Mandible	NHMW	*Ursus americanus*	64974		3	Adult		
Mandible	NMB	*Ursus americanus*	BA-8387		3	Adult	m	Zoo Basel
Mandible	NMB	*Ursus americanus*	BA-9151		3	Adult	f	Zoo Basel
Mandible	NMB	*Ursus americanus*	BA-9608	5 y	3	Adult	f	Zoo Basel
Mandible	NMB	*Ursus americanus*	BA-C.2189		3	Adult	f	Newfoundland
Mandible	NMB	*Ursus americanus*	BA-C.III.475	5 y	3	Adult	f	
Mandible	NMBE	*Ursus americanus*	BE-1021778	5 y	3	Adult	m	Canada
Mandible	NMBE	*Ursus americanus*	BE-1042654	7 y	3	Adult	f	Canada
Mandible	NMBE	*Ursus americanus*	BE-1042659		3	Adult		USA
Mandible	RBIN	*Ursus americanus*	KBIN-1139		3	Adult	m	Zoo Brussels
Mandible	RBIN	*Ursus americanus*	KBIN-1139 B		3	Adult	f	Zoo Brussels
Mandible	RBIN	*Ursus americanus*	KBIN-3384	7 y	3	Adult	m	Solvay
Mandible	RBIN	*Ursus americanus*	KBIN-40169		3	Adult		
Mandible	RBIN	*Ursus americanus*	KBIN-40170		3	Adult		
Mandible	RBIN	*Ursus americanus*	KBIN-40171		3	Adult		
Mandible	RBIN	*Ursus americanus*	KBIN-40172		3	Adult		
Mandible	NMB	*Ursus americanus*	BA-2962	5 y	5	Adult	m	
Mandible	NHMW	*Ursus arctos*	983		1	Juvenile		
Mandible	NHMW	*Ursus arctos*	2579		1	Juvenile		
Mandible	NHMW	*Ursus arctos*	2580		1	Juvenile		
Mandible	NMBE	*Ursus arctos*	BE-1042348	3 m	1	Juvenile	m	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042350	5 m	1	Juvenile		Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042357	4 m	1	Juvenile	m	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042358	4 m	1	Juvenile	f	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042405	4 w	1	Juvenile		Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042406		1	Juvenile	m	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042407		1	Juvenile	f	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042408	1.5 m	1	Juvenile	f	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042412	4 w	1	Juvenile		Tierpark Dhälhölzli Berne
Mandible	NMB	*Ursus arctos*	BA-8221	8 m	2	Juvenile		
Mandible	NMB	*Ursus arctos*	BA-C.III.351		2	Juvenile		
Mandible	NMBE	*Ursus arctos*	BE-1042347	6 m	2	Juvenile	m	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042349	6 m	2	Juvenile		Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042373	10 m	2	Juvenile	m	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042375	11 m	2	Juvenile	f	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042380	11 m	2	Juvenile	m	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042386	9 m	2	Juvenile	f	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042416	9 m	2	Juvenile	m	Tierpark Dhälhölzli Berne
Mandible	RBIN	*Ursus arctos*	1136 C		3	Adult	f	Zoo Brussels
Mandible	NMB	*Ursus arctos*	BA-10823		3	Adult	f	Finland
Mandible	NMB	*Ursus arctos*	BA-10870	8 y	3	Adult	f	
Mandible	NMB	*Ursus arctos*	BA-6541		3	Adult		Berne
Mandible	NMB	*Ursus arctos*	BA-6542	12 m	3	Adult		Berne
Mandible	NMB	*Ursus arctos*	BA-C.1565		3	Adult	f	Berne
Mandible	NMB	*Ursus arctos*	BA-C.III.363		3	Adult	f	
Mandible	NMB	*Ursus arctos*	BA-C.III.999		3	Adult	m	
Mandible	NMBE	*Ursus arctos*	BE-1030174		3	Adult	f	
Mandible	NMBE	*Ursus arctos*	BE-1042355	2 y	3	Adult	f	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042361	2 y	3	Adult	f	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042363	3 y	3	Adult	f	Tierpark Dhälhölzli Berne

Tabelle A1 cont.

Mandible	NMBE	*Ursus arctos*	BE-1042369	5 y	3	Adult	m	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042372	2 y	3	Adult	f	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042376	5 y	3	Adult	m	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042379	11 y	3	Adult	m	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042381	2 y	3	Adult	m	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042383	3 y	3	Adult	m	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042384		3	Adult	f	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042387	1 y 1m	3	Adult	m	
Mandible	NMBE	*Ursus arctos*	BE-1042388	1.5 y	3	Adult	m	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042390	2.5 y	3	Adult	f	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042392		3	Adult	f	
Mandible	NMBE	*Ursus arctos*	BE-1042394	4 y	3	Adult	f	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042396	2 y	3	Adult	f	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042399		3	Adult	f	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042418		3	Adult	m	
Mandible	NMBE	*Ursus arctos*	BE-1042425	1.5 y	3	Adult	f	
Mandible	NMBE	*Ursus arctos*	BE-1042426	3 y	3	Adult	f	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042553		3	Adult	f	Menagerie
Mandible	NMBE	*Ursus arctos*	BE-1042556	5 y	3	Adult	f	Alaska
Mandible	NMBE	*Ursus arctos*	BE-1047854		3	Adult	m	Graubünden
Mandible	NMBE	*Ursus arctos*	BE-1054540		3	Adult	m	Alaska
Mandible	NMBE	*Ursus arctos*	BE-1054620		3	Adult	f	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1065825	5 y	3	Adult	m	Tierpark Dhälhölzli Berne
Mandible	NMSG	*Ursus arctos*	St. G 3		3	Adult		
Mandible	ZMUZH	*Ursus arctos*	ZM10308		3	Adult		
Mandible	ZMUZH	*Ursus arctos*	ZM15198	2 y	3	Adult		
Mandible	NMB	*Ursus arctos*	BA-C.2470	8 y	4	Adult	m	North America
Mandible	NMBE	*Ursus arctos*	BE-1030173		4	Adult	m	Zoo Warsaw
Mandible	NMBE	*Ursus arctos*	BE-1030241	22 y	4	Adult	m	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042374	13 y	4	Adult	f	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042393	27 y	4	Adult	f	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042410	15 y	4	Adult	m	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042554		4	Adult		Canada
Mandible	NMBE	*Ursus arctos*	BE-1042343		5	Adult	m	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042366	23 y	5	Adult	f	Hungary
Mandible	NMBE	*Ursus arctos*	BE-1042391	27 y	5	Adult	f	Romania
Mandible	NMBE	*Ursus arctos*	BE-1042395		5	Adult	m	
Mandible	NMBE	*Ursus arctos*	BE-1042398	30 y	5	Adult	m	Tierpark Dhälhölzli Berne
Mandible	NMBE	*Ursus arctos*	BE-1042413		5	Adult	m	
Mandible	NMBE	*Ursus arctos*	BE-1042414	32 y	5	Adult	m	Bosnia
Mandible	NMBE	*Ursus arctos*	BE-1042417		5	Adult	f	Zoo Warschau
Mandible	NMBE	*Ursus arctos*	BE-1042555		5	Adult	m	Alaska
Mandible	NMBE	*Ursus arctos*	BE-1042563		5	Adult	f	Graubünden
Mandible	NMBE	*Ursus arctos*	BE-1054621		5	Adult	m	Mongolia
Mandible	NHMW	*Ursus maritimus*	22741		2	Juvenile		
Mandible	NHMW	*Ursus maritimus*	1322		3	Adult		
Mandible	NHMW	*Ursus maritimus*	1366		3	Adult		
Mandible	NHMW	*Ursus maritimus*	5320		3	Adult		
Mandible	NHMW	*Ursus maritimus*	5321		3	Adult		
Mandible	NHMW	*Ursus maritimus*	7138		3	Adult		
Mandible	NHMW	*Ursus maritimus*	7140		3	Adult		
Mandible	NHMW	*Ursus maritimus*	7141		3	Adult		
Mandible	NHMW	*Ursus maritimus*	7142		3	Adult		
Mandible	NHMW	*Ursus maritimus*	7143		3	Adult		

Tabelle A1 cont.

Mandible	NHMW	*Ursus maritimus*	7144		3	Adult		
Mandible	NHMW	*Ursus maritimus*	7145		3	Adult		
Mandible	NHMW	*Ursus maritimus*	7147		3	Adult		
Mandible	NHMW	*Ursus maritimus*	7148		3	Adult		
Mandible	NHMW	*Ursus maritimus*	7149		3	Adult		
Mandible	NHMW	*Ursus maritimus*	7225		3	Adult		
Mandible	NHMW	*Ursus maritimus*	7794		3	Adult		
Mandible	NHMW	*Ursus maritimus*	12691		3	Adult		
Mandible	NHMW	*Ursus maritimus*	13176		3	Adult		
Mandible	NHMW	*Ursus maritimus*	13329		3	Adult		
Mandible	NMB	*Ursus maritimus*	BA-10286		3	Adult		Basel
Mandible	NMB	*Ursus maritimus*	BA-10302	8 y	3	Adult	f	Basel
Mandible	NMB	*Ursus maritimus*	BA-6548		3	Adult		
Mandible	NMB	*Ursus maritimus*	BA-8272		3	Adult		Greenland
Mandible	NMB	*Ursus maritimus*	BA-9985		3	Adult		
Mandible	NMB	*Ursus maritimus*	BA-C.1294		3	Adult	m	Basel
Mandible	NMB	*Ursus maritimus*	BA-C.3270	5 y	3	Adult		
Mandible	NMB	*Ursus maritimus*	BA-C.4045		3	Adult	m	
Mandible	NMB	*Ursus maritimus*	BA-C.III.146		3	Adult		
Mandible	NMB	*Ursus maritimus*	BA-n.N.039		3	Adult		
Mandible	NMBE	*Ursus maritimus*	BE-1049101		3	Adult	m	Greenland
Mandible	RBIN	*Ursus maritimus*	KBIN-1133 C		3	Adult	m	
Mandible	RBIN	*Ursus maritimus*	KBIN-1133 F		3	Adult	f	Zoo Brussels
Mandible	RBIN	*Ursus maritimus*	KBIN-1134		3	Adult	m	Zoo Brussels
Mandible	RBIN	*Ursus maritimus*	KBIN-1134 B		3	Adult	f	
Mandible	RBIN	*Ursus maritimus*	KBIN-7551		3	Adult	m	
Mandible	RBIN	*Ursus maritimus*	KBIN-7553		3	Adult	f	
Mandible	RBIN	*Ursus maritimus*	KBIN-7555		3	Adult	m	
Mandible	RBIN	*Ursus maritimus*	KBIN-7556		3	Adult	m	
Mandible	NMSG	*Ursus maritimus*	St.G1		3	Adult		
Mandible	NHMW	*Ursus maritimus*	2568		4	Adult		
Mandible	NMB	*Ursus maritimus*	BA-10247	8 y	4	Adult	m	Basel
Mandible	NMB	*Ursus maritimus*	BA-10248	8 y	4	Adult		
Mandible	NMBE	*Ursus maritimus*	BE-1042675		4	Adult	f	Zoo Basel
Mandible	Montbéliard	*Ursus spelaeus*	MCP-2003 01-NB		1	Juvenile		Gondenans les Moulins
Mandible	NHMW	*Ursus spelaeus*	Baby		2	Juvenile		
Mandible	Montbéliard	*Ursus spelaeus*	MCP-2003 01 14		2	Juvenile	f	Gondenans les Moulins
Mandible	IfPEN	*Ursus spelaeus*	Sp.72/43		2	Juvenile		
Mandible	NHMW	*Ursus spelaeus*	2724		3	Adult		Salzhofenhöhle
Mandible	NHMW	*Ursus spelaeus*	5325		3	Adult		
Mandible	NHMW	*Ursus spelaeus*	5333		3	Adult		
Mandible	IfPEN	*Ursus spelaeus*	GL77/1115a&b		3	Adult		
Mandible	IfPEN	*Ursus spelaeus*	GL77/1119a&b		3	Adult		
Mandible	IfPEN	*Ursus spelaeus*	GL77/1122&1130		3	Adult		
Mandible	IfPEN	*Ursus spelaeus*	GL77/1127&1105		3	Adult		
Mandible	Montbéliard	*Ursus spelaeus*	MCP-2003 01 47		3	Adult	f	Gondenans les Moulins
Mandible	Montbéliard	*Ursus spelaeus*	MCP-2003 01-11		3	Adult		Gondenans les Moulins
Mandible	Montbéliard	*Ursus spelaeus*	MCP-2003 01-289		3	Adult	f	Gondenans les Moulins
Mandible	Montbéliard	*Ursus spelaeus*	MCP-2003 01-44		3	Adult	m	Gondenans les Moulins
Mandible	Montbéliard	*Ursus spelaeus*	MCP-2003 01-48		3	Adult	m	Gondenans les Moulins
Mandible	Montbéliard	*Ursus spelaeus*	MCP-2003 01-66		3	Adult	f	Gondenans les Moulins
Mandible	Montbéliard	*Ursus spelaeus*	Ro85 518-19		3	Adult		Gondenans les Moulins
Mandible	IfPEN	*Ursus spelaeus*	Sp.72/3		3	Adult		
Mandible	NHMW	*Ursus spelaeus*	5317		4	Adult		

Tabelle A1 cont.

Mandible	NMB	*Ursus spelaeus*	BA-9.166		4	Adult		Greenland
Mandible	NMB	*Ursus spelaeus*	BA-jf.775		4	Adult	m	Gondenans les Moulins
Mandible	Montbéliard	*Ursus spelaeus*	MCP-2003 01-12		4	Adult		Gondenans les Moulins
Mandible	Montbéliard	*Ursus spelaeus*	MCP-2003 01-145		4	Adult	m	Mancenans
Mandible	Montbéliard	*Ursus spelaeus*	MCP-2003 01-45		4	Adult	m	Gondenans les Moulins
Mandible	Montbéliard	*Ursus spelaeus*	MCP-2003 01-68		4	Adult		Gondenans les Moulins
Mandible	RBIN	*Ursus spelaeus*	RBIN 2172		4	Adult	f	Goyet Cave B5
Mandible	NHMW	*Ursus spelaeus*	XXVII.3		4	Adult		
Mandible	Montbéliard	*Ursus spelaeus*	MCP-2003 01 15		5	Adult	m	Gondenans les Moulins
Mandible	Montbéliard	*Ursus spelaeus*	MCP-2003 01-142		5	Adult	m	Mancenans
Mandible	Montbéliard	*Ursus spelaeus*	MCP-2003 01-65		5	Adult	m	Gondenans les Moulins
Mandible	RBIN	*Ursus spelaeus*	RBIN 2207		5	Adult	m	Goyet Cave B4
Mandible	NMSG	*Ursus spelaeus*	St. G Pr 6		5	Adult	m	
Mandible	NMSG	*Ursus spelaeus*	St.G 2		5	Adult	m	
Mandible	NHMW	*Ursus spelaeus*	XXVII.2		5	Adult		

Table A2. Angular comparison between paired regression vectors of the different species examined. The vectors were used with their original signs (possible range of angles 0 to 180°). Significance values are given in brackets and significant results are in bold.

	U. americanus	*U. arctos*	*U. maritimus*
Cranium			
U. americanus			
U. arctos	**33.628 (<.00001)**		
U. maritimus	**53.952 (<.00001)**	**65.103 (0.00001)**	
U. spelaeus	**43.275 (<.00001)**	**38.232 (<.00001)**	**62.646 (<.00001)**
Mandible			
U. americanus			
U. arctos	79.685 (0.20125)		
U. maritimus	77.254 (0.15010)	**51.794 (0.00064)**	
U. spelaeus	81.206 (0.23786)	**33.036 (<.00001)**	**48.623 (0.00022)**

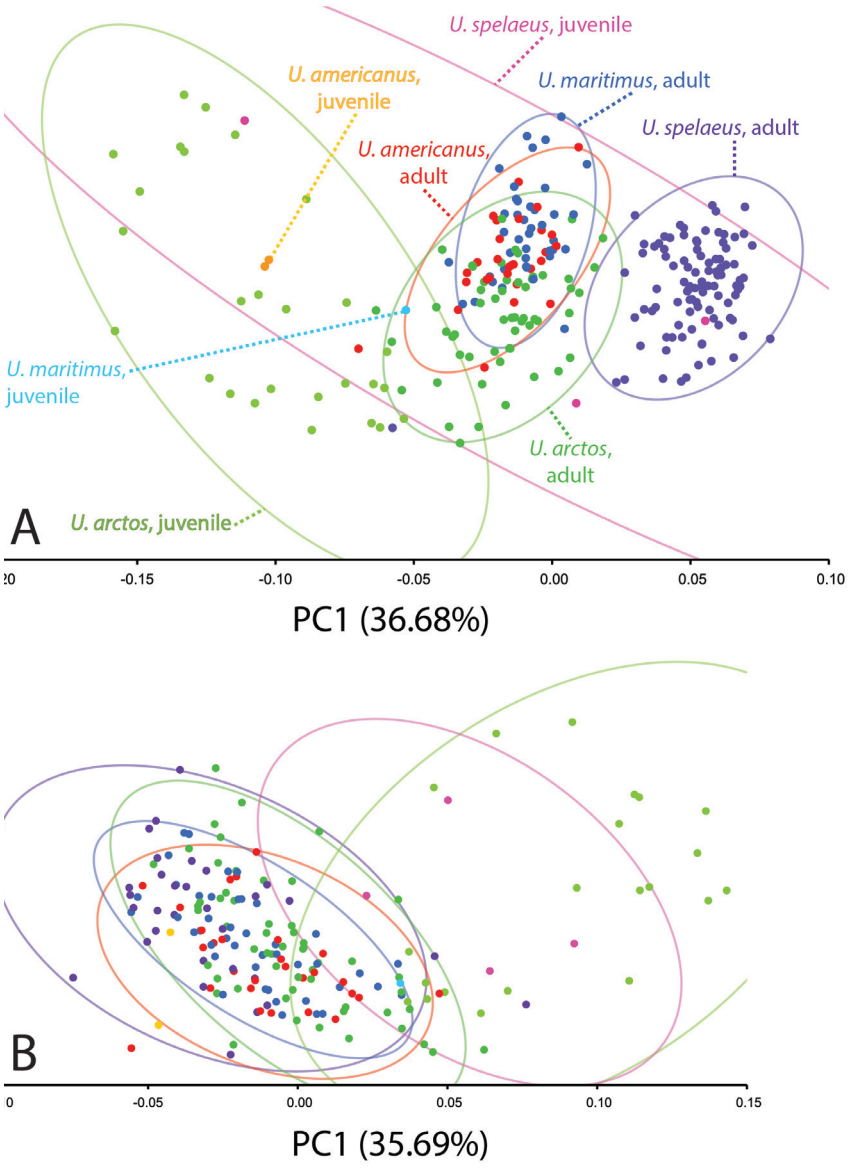

Figure A1. Principal component analysis of cranial (A) and mandibular (B) shape variation in juvenile and adult bear species. Ellipses represent the 95% confidence interval of the age stages (juvenile and adult) within species.

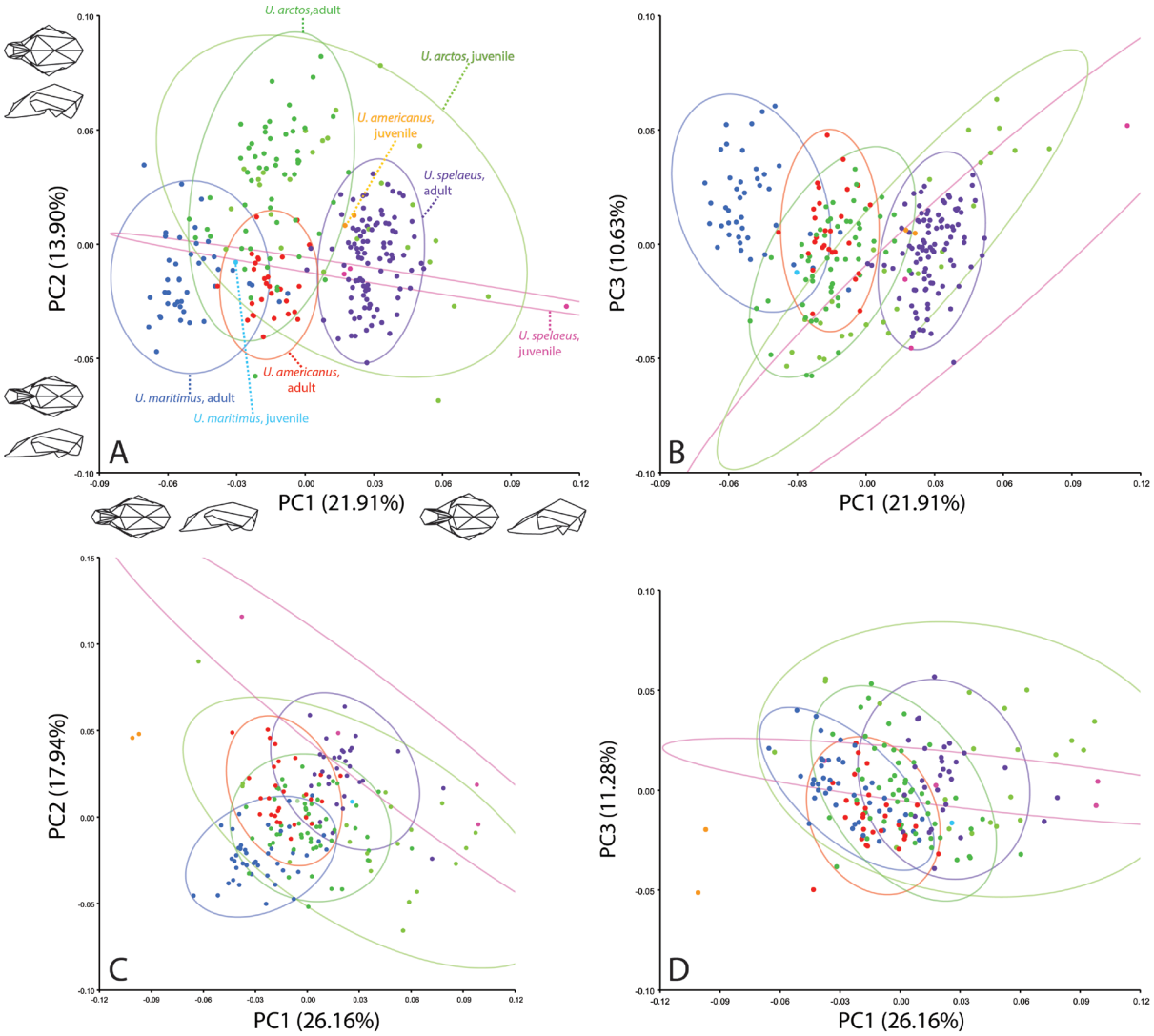

Figure A2. Principal component analysis of the non-allometric component of cranial (A,B) and mandibular (C,D) shape variation in juvenile and adult bear species. Ellipses represent the 95% confidence interval of the age stages (juvenile and adult) within species. Cranial and mandibular models represent extreme shape forms on PC1 and PC3.